Pharmacological Characterizations of Adrenergic Receptors in Human Adipocytes

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ABSTRACT Three types of adrenergic receptors, beta, alpha-1, and alpha-2, were identified in human adipocytes, isolated from properitoneal adipose tissue, using both the binding of radioactive ligands and the effects of adrenergic agents on receptor-specific biochemical responses. Adrenergic binding studies showed the following results: [^{3}H]dihydroalprenolol binding (beta adrenergic) B_{max} 280 fmol/mg protein, K_D 0.38 nM; [^{3}H]para-aminoclonidine binding (alpha-2 adrenergic) B_{max} 166 fmol/mg protein, K_D 0.49 nM; [^{3}H]WB 4101 binding (alpha-1 adrenergic) B_{max} 303 fmol/mg protein, K_D 0.86 nM. In adipocytes from subcutaneous adipose tissue, [^{3}H]dihydroergocryptine binding indicated the presence of alpha-2 but not alpha-1 receptors.

Beta and alpha-2 adrenergic receptors appeared to be positively and negatively coupled to adenylate cyclase, respectively. Cells or cell membranes were incubated with epinephrine (10 μM) alone and in combination with the antagonists yohimbine (alpha-2) and prazosin (alpha-1). Epinephrine alone prompted a modest increase in adenylate cyclase activity, cyclic AMP, and glycerol release, an index of lipolysis. Yohimbine (0.1 μM) greatly enhanced these actions whereas prazosin was without effect. The beta agonist, isoproterenol, stimulated glycerol release, whereas the alpha-2 agonist, clonidine, inhibited lipolysis and cyclic AMP accumulation. To assess further alpha-1 receptors, cells were incubated with [^{32}P]phosphate and epinephrine (10 μM) alone and in combination with prazosin and yohimbine. Epinephrine alone caused a three- to fourfold increase in ^{32}P incorporation into phosphatidylinositol. Prazosin (0.1 μM) blocked this action whereas yohimbine (0.1 μM) was without effect. Thus, in a homogeneous cell preparation, the human adipocyte appears to have three different adrenergic receptors, each of which is coupled to a distinct biochemical response.

Dr. J. A. García-Saínz is an international postdoctoral fellow sponsored by the National Institutes of Health. Dr. B. B. Hoffman is a fellow of the Medical Research Council of Canada. Dr. R. J. Lefkowitz is an investigator of Howard Hughes Medical Institute.

Received for publication 25 July 1980 and in revised form 3 October 1980.

INTRODUCTION

Over 30 yr ago, Ahlquist (1) proposed that the cellular responses to epinephrine, norepinephrine, and the synthetic catecholamine, isoproterenol, could be divided into two groups, which he designated alpha and beta, according to their potencies in interacting with the proposed receptors. With alpha-mediated responses, epinephrine was most potent and isoproterenol least potent; with beta-mediated responses, isoproterenol was most active and norepinephrine least so. Subsequently, Lands et al. (2) suggested that beta receptors could be further divided into two groups, beta-1 and beta-2. Both beta-1 and beta-2 receptors appear to stimulate adenylate cyclase leading to an increase in cyclic AMP and to an expression of the tissue-specific function. More recently, alpha receptors have been divided into alpha-1 and alpha-2 subtypes (3–5). Although the initial classification was based on anatomic localization, it appears that a classification related to function is more reasonable (3, 4). The means by which alpha adrenergic stimulation affects cellular function is not completely understood. In several systems, stimulation of alpha-1 adrenoceptors increases phosphatidylinositol turnover and calcium gating or mobilization whereas activation of alpha-2

sites decreases cyclic AMP through adenylate cyclase inhibition (4, 5).

The human adipocyte is of interest because it is a single cell type that has both alpha and beta receptors. Catecholamines seem to be physiologically important mediators for lipid mobilization in these cells (6–13). Beta adrenergic stimulation increases adenylate cyclase activity, raises cyclic AMP, and enhances the cyclic AMP-dependent processes such as lipolysis, whereas alpha adrenergic activation, in general, has opposite effects (6–13). These observations support the hypothesis put forward by Robison et al. (14) that some cell types have both alpha and beta adrenoceptors that mediate opposite effects on cyclic AMP and cell function. The studies reported here were carried out to characterize further the adrenergic receptors of human adipocytes and the metabolic functions they subserve by using selective agonists and antagonists.

METHODS

Cell preparation. Abdominal adipose tissue samples were obtained from subjects undergoing elective abdominal surgery. Informed consent, approved by the institution's Human Experimentation Committee, was obtained from each subject. Because all subjects were infused with 5% dextrose during surgery, they were considered to be in the fed state. Fat cells were obtained by collagenase digestion of adipose tissue (15). For experiments involving measurement of cyclic AMP and glycerol release, cells were isolated and then suspended in Krebs bicarbonate buffer containing 4% bovine serum albumin and 2.5 mM glucose. Flasks containing portions of cell suspensions were incubated with gentle shaking in an atmosphere of 95% O_2 and 5% CO_2. Fat cell ghosts were prepared by hypotonic lysis at 10°C with 5 mM Tris HCl buffer (pH 7.5, 25°C) containing 2.5 mM $MgCl_2$ and 1 mM EDTA as described by Birnbaumer et al. (16).

Radioligand binding assays. Binding studies with dihydroalprenolol, *p*-aminoclonidine, and WB4101 were performed using membranes prepared from adipocyte ghosts stored at −20°C for 1–4 d. The ghosts were homogenized (Tissumizer, Tekmar Co., Cincinnati, Ohio; setting 8, 30 s) in 35 ml of ice-cold 50 mM Tris HCl buffer (pH 8.0 at 25°C). This homogenate was centrifuged (10 min at 49,000 *g*) and then resuspended in buffer at a concentration of ~0.10 mg protein/ml.

Beta adrenergic receptors were assayed as described previously (17). Total binding was determined with one set of incubation tubes, which contained 970 μl of the membrane suspension in 50 mM Tris HCl buffer (pH 8.0) and 20 μl of increasing concentrations of [^{3}H]dihydroalprenolol diluted in 5 mM HCl. The final concentration of [^{3}H]dihydroalprenolol ranged from about 0.2 to 4.0 nM. A parallel set of incubations containing 0.3 μM propranolol was used to determine nonspecific binding. Specific binding is taken as the difference between total and nonspecific binding. After a 25-min incubation at 23°C, the suspensions were filtered through Whatman GF/B glass filter paper (Whatman, Inc., Clifton, N. J.) using a manifold (Brandel Cell Harvester, Biomedical Research and Development), which filters 24 samples simultaneously. The tubes and filter paper were washed with 3 and 6 ml of cold buffer, and then radioactivity retained by the filter paper was determined for each sample by scintillation spectroscopy.

Alpha adrenergic receptor assays with [^{3}H]WB4101 as the radioligand (concentration range, 0.3–4 nM) were performed as described by U'Prichard et al. (18), and 100 μM norepinephrine was used to determine nonspecific binding. Alpha-2 adrenergic receptor binding was determined by the procedure of Rouot and Snyder (19) using [^{3}H]para-aminoclonidine as the radioligand (concentration range, 0.1–2.0 nM), and 10 μM norepinephrine was used to determine nonspecific binding. The pH of the buffer was 7.4 for the alpha-2 adrenergic receptor binding studies.

All studies were done using adipocytes isolated from properitoneal adipose tissue at the University of Missouri except for the studies with [^{3}H]dihydroergocryptine binding, which were performed using membranes obtained from adipocytes isolated from subcutaneous adipose tissue at Duke University. The following procedure was used for studies on dihydroergocryptine binding. Adipocytes that had been washed three times were homogenized at room temperature with 10 strokes of a Teflon pestle in buffer containing 0.25 sucrose, 10 mM Tris HCl, and 1 mM EDTA at pH 7.4. The lysate was spun at 39,000 *g* for 10 min at 4°C. The pellet was resuspended in 50 mM Tris HCl, 10 mM $mgCl_2$ (pH 7.5), and spun down again at 39,000 *g* for 10 min. The resulting pellet was resuspended in 50 mM Tris HCl, 10 mM $MgCl_2$ (pH 7.5) at a protein concentration of 2 mg/ml, frozen, and stored under liquid nitrogen until used within 1 wk. Assay conditions for [^{3}H]dihydroergocryptine binding and computer modeling techniques were as previously described (5).

Cyclic AMP, glycerol, and adenylate cyclase assays. For the determination of cyclic AMP, test substances were incubated in 1 ml of cell suspension for 20 min. At the end of incubation, the contents of each flask were mixed with 1 ml of 0.6 N perchloric acid, and after removal of the protein, the perchloric acid was neutralized with potassium hydroxide. After centrifugation to remove the precipitated potassium perchlorate, the cyclic AMP content of the supernate was then estimated by radioimmunoassay using a modification of Steiner et al. (20). Cyclic AMP concentration is expressed as picomoles/10^6 cells.

To evaluate effects on lipolysis, test substances were incubated for 4 h with 1 ml of cells in each of three flasks. After incubation, cell-free filtrates were prepared and analyzed for glycerol using the method of Garland and Randle (21). The concentration of glycerol is expressed as micromoles/10^6 cells in 4 h. For adenylate cyclase assay, membranes prepared by the method of Birnbaumer et al. (16) were resuspended in sufficient buffer to provide a concentration of 4 mg protein/ml. Adenylate cyclase activity was determined by the method of Salomon et al. (22) at pH 8.0. Enzyme activity is expressed as picomoles of cyclic AMP formed per milligram protein per minute.

Phospholipid analysis. The incorporation of [^{32}P]phosphate into phospholipids was measured as previously described (5, 23). Fat cells were incubated in plastic bottles containing 3 ml of phosphate-free medium and [^{32}P]phosphoric acid at a concentration of 10–15 μCi/ml. 1 ml of packed cells was added to each bottle. After 1-h incubation, the medium was aspirated and lipids extracted with chloroform/methanol (2:1). Phospholipids were absorbed on silicic acid granules and removed from the silicic acid with methanol. Major phospholipids were separated by one-dimensional thin-layer chromatography on glass plates coated with silica gel H (Merck Chemical Div., Merck & Co., Rahway, N. J.) containing 1 g of magnesium acetate for each 40 g silica. The solvent system was chloroform/methanol/water/28%

NH_4OH (130:70:5:5). This procedure was suitable for the separation of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Phosphatidic acid was not separated from phosphatidylserine, which migrated only slightly above the origin. Although the amount of phosphatidylserine plus phosphatidic acid may be overestimated by the presence of some contaminants at the origin, nearly all the radioactivity corresponded with phosphatidic acid. The same comment is applicable to phosphatidylinositol caused by incomplete separation from sphingomyelin. The incorporation of label into phosphatidylserine or sphingomyelin is less than 10% of that of phosphatidic acid or phosphatidylinositol in rat adipocytes (23). There is no effect of catecholamines on the labeling of phosphatidylserine or sphingomyelin in rat adipocytes (23). Lipids were visualized by iodine vapor staining. The phosphorus content of phospholipids was determined by a micro modification of the procedure of Bartlett (24) after acid hydrolysis of silica gel scraping containing each individual phospholipid.

Materials. [^{3}H]dihydroalprenolol (49.0 Ci/mmol), [^{3}H]para-aminoclonidine (52 Ci/mol), [^{3}H]WB4101 (2,6-dimethoxyphenyloxyethyl aminomethyl-1, 4-benzodioxane, 24 Ci/mmol), [^{3}H]dihydroergocriptine (32 Ci/mmol), [^{3}H]-cyclic AMP (30 mCi/mmol) and (α^{32}P) ATP (15 Ci/mmol), and [^{32}P]phosphoric acid (carrier-free, 99% radionuclide purity) were obtained from New England Nuclear (Boston, Mass.).

The following drugs were kindly donated by the indicated company: (−)-propranolol (Ayerst, Laboratories, New York), (−)-isoproterenol (Sterling-Winthrop Research Institute, Rensselaer, N. Y.), phentolamine (Ciba-Geigy Corp., Pharmaceuticals Div., Summit, N. J.), prazosin (Pfizer, Chemical Div., Pfizer Inc., New York), clonidine (Boehringer Ingelheim, Elmsford, N. Y.), and methoxamine (Burroughs-Wellnorepinephrine, and yohimbine were obtained from Sigma Chemical Co. (St. Louis, Mo.). Adenosine deaminase was obtained from Boehringer Mannheim, Biochemicals (Indianapolis, Ind.).

RESULTS

Binding of radioligands to adrenergic receptors of the human adipocyte. The three radioligands, [^{3}H]-dihydroalprenolol, [^{3}H]para-aminoclonidine, and [^{3}H]-WB4101 demonstrated specific binding to adipocyte membranes prepared from properitoneal tissue suggesting the presence of beta, alpha-2, and alpha-1 adrenergic receptor binding sites. Saturation experiments for each ligand indicated the presence of a single class of high affinity sites (Figs. 1 and 2). The K_D and B_{max} values were calculated from a least-squares analysis plotted as bound/free vs. bound (25). The K_D and B_{max} values for membranes prepared from adipose tissue from five patients are given in Table I. The binding of [^{3}H]dihydroalprenolol to beta adrenergic receptors had a mean K_D of 0.38 nM and a B_{max} (receptor density) of 280 fmol/mg protein. In the same membrane preparation the binding of [^{3}H]para-aminoclonidine to alpha-2 receptors had a K_D of 0.49 nM and a B_{max} of 166 fmol/mg protein. Similarly, [^{3}H]WB4101 appeared to label alpha-1 adrenergic receptors with a K_D of 0.86 and a B_{max} of 303 fmol/mg protein. The density of beta adrenergic receptors was remarkably constant in five patients, whereas alpha-2

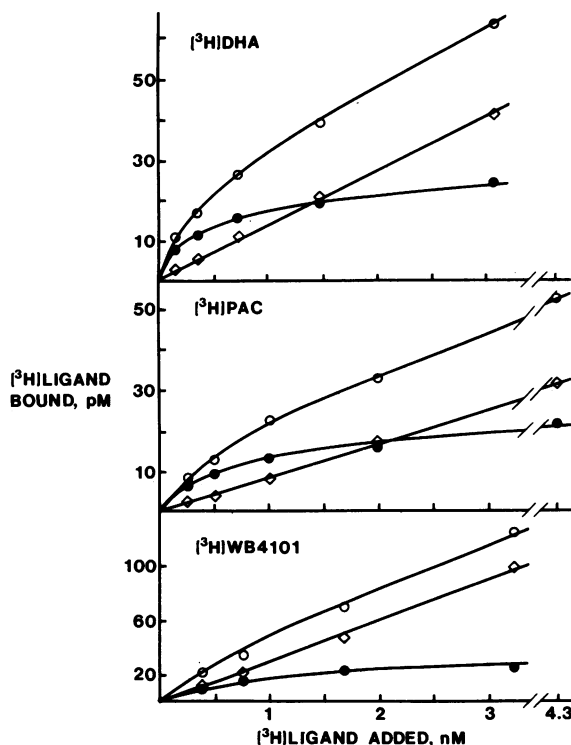

FIGURE 1 Saturation of specific radioligand receptor binding sites. Membranes derived from adipocytes were incubated for 25 min at 23°C with increasing concentrations of each radioligand: upper panel, [^{3}H]dihydroalprenolol (DHA) (beta receptors); middle panel, [^{3}H]para-aminoclonidine (PAC) (alpha-2 receptors); and lower panel, [^{3}H]WB4101 (alpha-1 receptors). The concentration of radioligand is plotted on the abscissa, and the concentration of bound radioligand is plotted on the ordinate. Specific binding (●) is the difference between the total binding (○) and nonspecific binding (◇). Results shown are from a representative experiment (number 705 in Table I) in which the protein concentration was 0.11 mg/ml.

and alpha-1 receptor densities were more variable. The ratio of dihydroalprenolol binding to that of p-aminoclonidine was 1.8±0.4 (mean±SEM) whereas the ratio of dihydroalprenolol to WB4101 binding was 1.0±0.2. For all three receptors, the K_D was relatively constant among the different preparations.

[^{3}H]dihydroergocryptine, an alpha antagonist, labels alpha-1 and alpha-2 receptors with equal affinity in a variety of systems (26). In membranes derived from subcutaneous human adipose tissue, [^{3}H]dihydroergocryptine binding is saturable and of high affinity. The K_D was 1.2 nM, and the B_{max} was 120±18 fmol/mg protein (n = 7). In an effort to determine which alpha-adrenergic receptor subtype(s) were present in these adipocyte membranes, competition curves of [^{3}H]-dihydroergocryptine were performed with several antagonists (26, 27) (Fig. 3). The alpha-2-selective antagonist yohimbine was much more potent (K_D

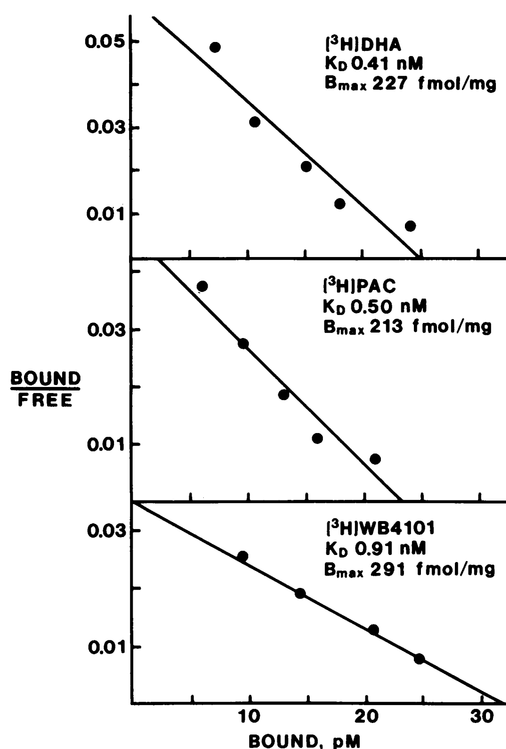

FIGURE 2 Rosenthal analysis of specific radioligand binding. The binding data presented in Fig. 1 were transformed according to Rosenthal. Specific binding (●) is plotted on the abscissa, and the ratio of specific binding to free radioligand is plotted on the ordinate. The concentration of binding sites (B_{max}) is the intercept on the abscissa, and the affinity (K_D) is the negative reciprocal of the slope.

TABLE I
Adrenergic Receptor Binding in Human Adipocyte Membranes

Preparation number	Beta adrenergic [3H]dihydroalprenolol		Alpha-2 adrenergic [3H]p-aminoclonidine		Alpha-1 adrenergic [3H]WB4101	
	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D
705	227	0.41	213	0.50	291	0.91
709	297	0.45	—	—	175	1.00
715	323	0.32	185	0.36	451	0.99
717	269	0.23	92	0.57	345	0.70
718	284	0.47	174	0.51	253	0.68
Mean	280	0.38	166	0.49	303	0.86
SEM	16	0.04	26	0.04	46	0.07

B_{max} (receptor density, femtomoles per milligram protein) and K_D (affinity, nanomolar) were determined for each receptor as described in Fig. 1. Each preparation is from a different patient.

= 8 nM, $n = 2$) than the alpha-1 selective antagonist prazosin (K_D = 4,600 nM, $n = 4$). Also, low concentrations of prazosin did not significantly inhibit [3H]-dihydroergocryptine binding suggesting that the alpha receptors of adipocytes from subcutaneous tissue contain predominantly alpha-2 receptors with very few alpha-1 receptors. In previous studies using [3H]-dihydroergocryptine, about 90% of the alpha receptors of the hamster adipocytes were found to be of the alpha-2 subtype (5). Similarly, alpha-1 receptors were not detected in adipocyte membranes from subcutaneous tissue using [3H]WB4101 as the radioligand (Bylund, D. B. Unpublished observations).

Alpha-2 adrenergic responses: depression of lypolysis, cyclic AMP, and adenylate cyclase activity. Cells were incubated with increasing concentrations of isoproterenol (pure beta), epinephrine (mixed alpha/beta), methoxamine (alpha-1) or clonidine (partial alpha-2), and glycerol release measured after 4-h incubation (Fig. 4). Isoproterenol stimulated lipolysis substantially, epinephrine was less potent, methoxamine had little or no effect, and clonidine depressed basal glycerol release (Fig. 4). In other experiments, epinephrine (10 μM) was incubated in the presence of increasing concentrations of the antagonist yohimbine (alpha-2), phentolamine (alpha-1 and alpha-2), or prazosin (alpha-1). At a concentration of 0.1 μM both yohimbine and phentolamine enhanced epinephrine stimulated lipolysis whereas prazosin even at 10 μM was without effect (Fig. 5).

Cells were incubated with clonidine and methoxamine, and, in other experiments, with epinephrine alone and in combination with varying concentrations of the antagonist yohimbine, phentolamine, and

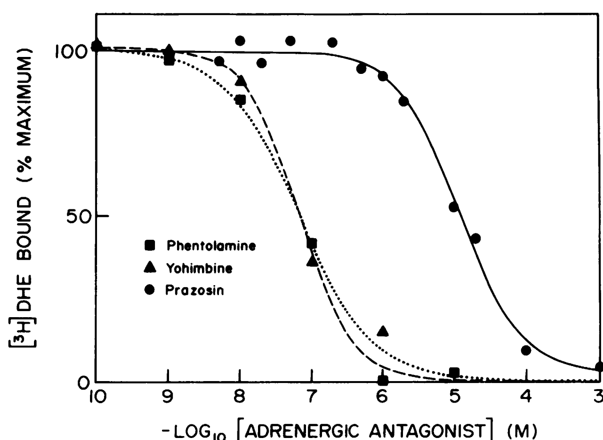

FIGURE 3 Competition curves of [3H]dihydroergocryptine (DHE) by alpha adrenergic antagonists. Membranes were prepared from adipocytes derived from subcutaneous fat. In this representative experiment, performed in duplicate, yohimbine was 500-fold more potent than prazosin indicating the predominant presence of alpha-2 receptors. The [3H]dihydroergocryptine concentration was 8 nM.

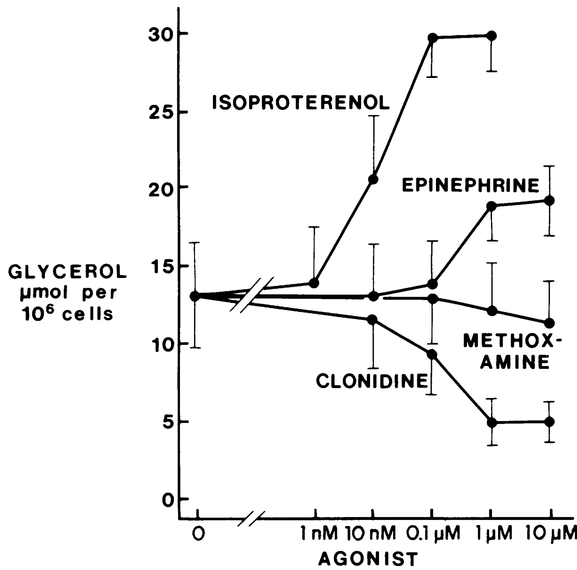

FIGURE 4 The effect of adrenergic agonist on glycerol release by human adipocytes. Cells were incubated for 4 h with increasing concentrations of the agonists shown. The values shown are the means±SEM of four experiments.

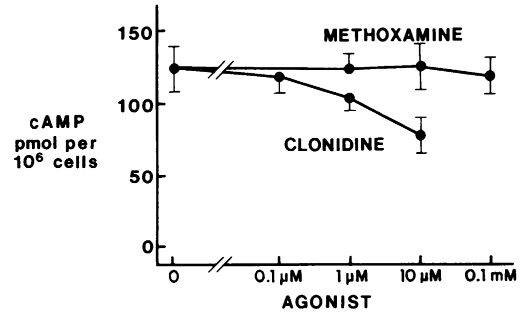

FIGURE 6 The effect of clonidine and methoxamine on basal cyclic AMP. Cells were incubated for 20 min with increasing concentrations of the agonists. The values are the means±SEM of four experiments in the presence of 0.5 μg/ml of adenosine deaminase and 0.1 mM theophylline.

prazosin. Cyclic AMP was estimated at the end of a 20-min incubation. Clonidine depressed basal cyclic AMP accumulation whereas methoxamine even at 0.1 mM was without effect (Fig. 6). Yohimbine and phentolamine both markedly enhanced epinephrine-stimulated cyclic AMP whereas prazosin was without effect (Fig. 7). Isoproterenol (10 μM) elevated cyclic AMP from a basal value of 55 to 9,980 pmol/10^5 cells (data not shown) whereas epinephrine 10 μM increased the nucleotide only to 90 pmol/10^6 cells. If the failure of epinephrine to elevate cyclic AMP to the same extent as isoproterenol were caused by concurrent stimulation of alpha-2 receptors, one would expect yohimbine and phentolamine to enhance the epinephrine stimulation and prazosin to have no effect. The results were as predicted and indicate the presence of alpha-2 catecholamine receptors, which inhibit both lipolysis and cyclic AMP accumulation (Figs. 5 and 6).

Fat cell ghosts were incubated for 15 min with epinephrine 10 μM alone and with increasing concentrations of yohimbine, phentolamine, and prazosin. None of the antagonists affected basal adenylate cyclase activity (data not shown). Both yohimbine and phentolamine enhanced the stimulating action of epinephrine on enzyme activity whereas prazosin was without effect (Fig. 8).

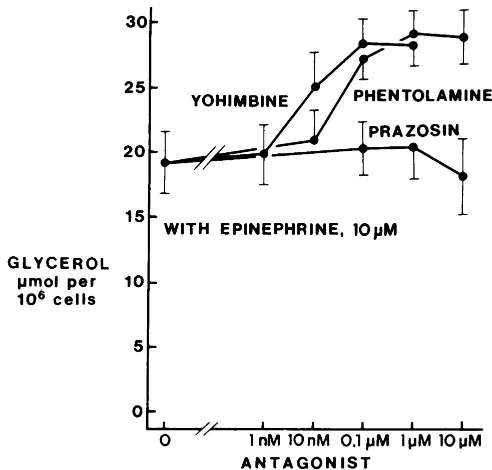

FIGURE 5 The effect of adrenergic antagonists on epinephrine-stimulated glycerol release. Cells were incubated for 4 h with epinephrine (10 μM) and increasing concentrations of the antagonists shown. The values shown are the means±SEM of four experiments.

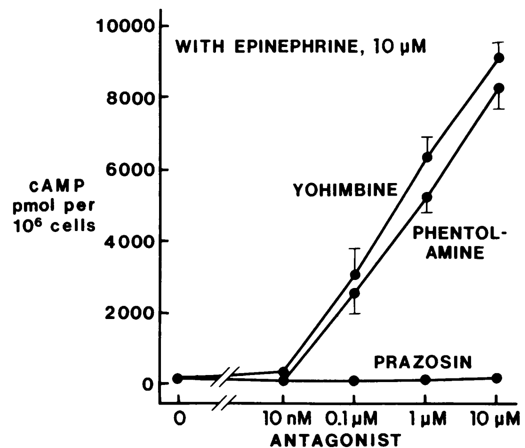

FIGURE 7 The effect of adrenergic antagonists on the cyclic AMP concentration of human adipocytes. Cells were incubated for 20 min with epinephrine and varying concentrations of the antagonists shown in the presence of 0.5 μg/ml of adenosine deaminase and 0.1 mM theophylline. The values are the means±SEM of four experiments.

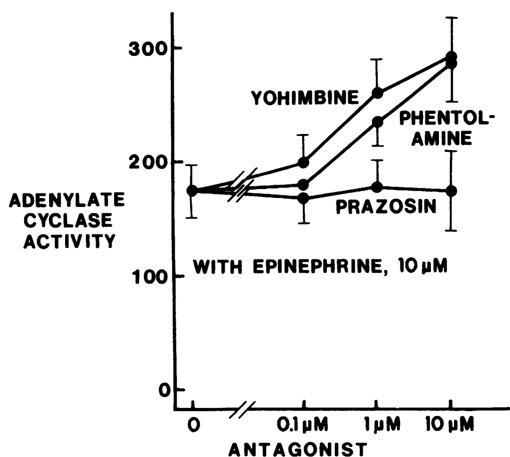

FIGURE 8 The effect of adrenergic antagonists on the adenylate cyclase activity (expressed as picamoles of cyclic AMP formed per milligram protein per minute) of human adipocyte membranes. Membranes were incubated for 10 min in Tris buffer containing [^{32}P]ATP, epinephrine, and varying concentrations of the antagonists shown. The values are the means±SEM of four experiments.

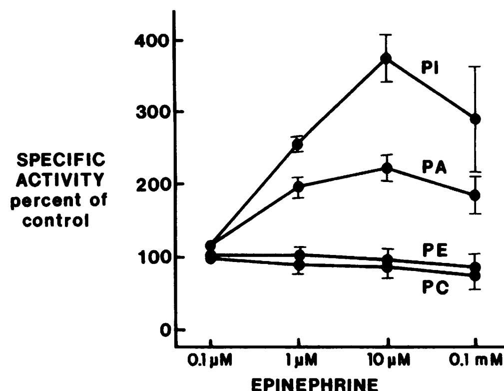

FIGURE 9 Stimulation of ^{32}P uptake into phosphatidic acid (PA) and phosphatidylinositol (PI) by epinephrine. Adipocytes were incubated with increasing concentrations of epinephrine. Basal values are given in Table II. The values given are the means±SEM of four paired experiments. There was little or no effect of epinephrine on phosphatidylcholine (PC) or phosphatidylethanolamine (PE) turnover.

Alpha-1 adrenergic response: stimulation of phospholipid turnover. The phospholipid composition and the incorporation of radioactive phosphate into major phospholipids in human fat cells were similar to those observed in rat and hamster adipocytes (5, 23). Under basal conditions, phosphatidic acid and phosphatidylserine contained 20±2% (mean and SEM of four experiments) of the total phosphate, phosphatidylinositol, and sphingomyelin, 15±1%, phosphatidylcholine, 28±1%, phosphatidylethanolamine 30±2%, and cardiolipin 7±1%. The incorporation of radioactive phosphate was mainly into phosphatidylcholine (67±11% of the total incorporation into phospholipids), phosphatidic acid plus phosphatidylserine (24±10%), and phosphatidylinositol (6±1%). Less incorporation was observed into phosphatidylethanolamine (3±1%) and cardiolipin (<1%). The values are the means±SEM of four experiments.

Epinephrine produced a dose-dependent increase in the incorporation of radioactive phosphate into phosphatidylinositol and phosphatidic acid (Fig. 9). A small decrease in the labeling of phosphatidylethanolamine and phosphatidylcholine was observed at concentrations of epinephrine above 10 µM (Fig. 9), whereas the labeling of cardiolipin was not affected by 10 µM epinephrine (Table II). The decrease in the labeling of phospholipids at high concentrations of epinephrine was previously observed in rat adipocytes and attributed to intracellular accumulation of free fatty acids (23). The pure beta adrenergic agent, isoproterenol, did not mimic the effect of epinephrine on the labeling of phosphatidylinositol and phosphatidic acid indicating that this effect of epinephrine is mediated through the stimulation of alpha receptors (Table II). Furthermore, the action of epinephrine on phosphatidylinositol labeling was actually greater in the presence of the beta adrenergic blocking agent, propranolol (Table II). Propranolol itself produced a small increase in labeling of phosphatidylinositol and phosphatidic acid (Table II). This effect of propranolol has been observed in the rat adipocyte (23), and in other tissues (28, 29) but seems to be caused by its local anesthetic properties rather than by its action as a beta blocker.

The stimulation by epinephrine of ^{32}P incorporation into phosphatidylinositol and phosphatidic acid was blocked by selective alpha adrenergic antagonists (Fig. 10). The potency order was prazosin > phentolamine > yohimbine, indicating that this action of epinephrine is mediated by the activation of alpha-1 adrenoceptors. Prazosin (1 µM), phentolamine (10 µM), and yohimbine (0.1 mM) each alone did not modify the labeling of phospholipids (Table II).

DISCUSSION

The principal finding of these studies is that a single type of cell, the human adipocyte, appears to have three different types of adrenergic receptors, each coupled to a distinct biochemical response: beta, activation of adenylate cyclase; alpha-2, inhibition of adenylate cyclase; and alpha-1, stimulation of phosphatidylinositol turnover. The data supporting this conclusion derive from two different types of experiments: (*a*) the binding of specific radioligands, and (*b*) the effects of pharmacological agents on receptor-specific biochemical responses.

Table II

Phospholipid Content and Effects of Various Agents of ^{32}P Uptake into Adipocyte Phospholipids

	Phosphatidic acid + phosphatidylserine	Phosphatidylinositol + spingomyelin	Phosphatidylcholine	Phosphatidylethanolamine	Cardiolipin
Phosphate content, μg	0.77	0.57	1.01	1.13	0.26
^{32}P uptake in counts per minute	6,540	2,120	30,210	980	300
	Effect of agents on ^{32}P counts per minute as percent control±SE				
Epinephrine, 10 μM	220±16	376±30	88±12	89±14	128±14
Prazosin, 1 μM	110±10	105±3	104±10	150±22	170±23
Phentolamine, 10 μM	112±14	121±7	84±1	117±18	175±25
Yohimbine, 100 μM	120±14	131±13	112±1	105±2	103±19
Isoproterenol, 10 μM	95	93	83	78	99
Propranolol, 30 μM	130	171	82	88	121
Propranolol, 30 μM + epinephrine, 20 μM	208	567	82	86	156

Human adipocytes (1 ml of packed cells) were incubated for 60 min in 4 ml of buffer containing 6% albumin and 10–15 μCi/ml of $[^{32}P]P_i$. The values are for four paired experiments except for those with propranolol or isoproterenol which represent the means of two experiments.

Whereas the beta effect of catecholamines has long been recognized as being mediated by the activation of adenylate cyclase, the mechanism of action of the alpha receptor has been controversial. We (6) and others (13) have obtained various evidence suggesting that the decrease in lipolysis and in cyclic AMP concentration resulting from alpha stimulation of the human adipocyte is mediated by the inhibition of adenylate cyclase. The present studies confirm these results and, in addition, define this alpha response as being of the alpha-2 subtype. A similar conclusion was also reached by Lafontan and Berlan (30) based on agonist inhibition of theophylline-induced lipolysis in human adipocytes and antagonism of clonidine action. It appears that the alpha-2 receptor in a number of other tissues and species may also be negatively coupled to adenylate cyclase (4, 5).

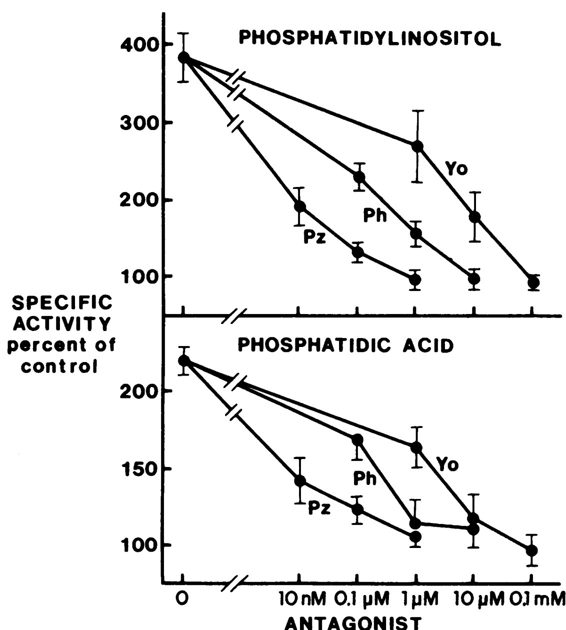

FIGURE 10 Inhibition of epinephrine-stimulated incorporation of ^{32}P into phsophatidylinositol and phosphatidic acid by alpha antagonists. Adipocytes were incubated with epinephrine (10 μM) alone or with varying concentrations of prazosin (Pz), phentolamine (Ph), and yohimbine (Yo). Basal values and those for the highest concentrations of each inhibitor in the absence of epinephrine are given in Table II. The values are the means±SEM of four experiments.

Alpha-1 stimulation in the human adipocyte results in the increased turnover of phosphatidylinositol and phosphatidic acid. It is thought that the increased turnover of these phospholipids in many tissues results in an increase in the cytoplasmic calcium ion concentration (4, 31, 32). For example, in the rat adipocyte alpha-1 adrenergic stimulation results both in an increase in phosphatidylinositol turnover and the inactivation of glycogen synthase (33). This inactivation can be mimicked by the divalent cation ionophore A23187 (34, 35). There is little effect of either alpha-1 adrenergic activation or the divalent cation ionophore A23187 on cyclic AMP accumulation and lipolysis in hamster or rat adipocytes (5, 36, 37). Michell and his associate (31, 32) have pointed out the remarkable correlation between these two processes and suggested that turnover of phosphatidylinositol is involved in some fashion in the gating or mobilization of calcium. However, the link between the ability of alpha-1 catecholamines to elevate cytosolic calcium in many

cells and increased turnover of phosphatidylinositol is not yet proven (31, 32).

Each of the radioligands used in the binding studies exhibited high affinity and saturable binding to a single class of binding sites. The affinity of each radioligand at its receptor binding site and the density of receptor binding sites found in the human adipocyte are similar to those in other adipocytes (5, 38). Preliminary results of studies in progress using unlabeled adrenergic agents are consistent with the conclusion that the radioligands are specifically labeling the appropriate receptors (Bylund, D. B. Unpublished data). For example, isoproterenol is more potent in inhibiting [^{3}H]-dihydroalprenolol binding than is either epinephrine or norepinephrine. Furthermore, prazosin, an alpha-1 antagonist, was considerably more potent in inhibiting [^{3}H]WB4101 binding than [^{3}H]para-aminoclonidine, whereas yohimbine, an alpha-2 antagonist, was more potent on [^{3}H]para-aminoclonidine binding. It is particularly noteworthy that whereas properitoneal and subcutaneous adipose tissue appear to have similar levels of alpha-2 receptors, the former has ~50% more alpha-1 than alpha-2, while alpha-1 receptors were not detected in the latter.

The ability to study the three adrenergic receptors of the human adipocyte on the basis of both binding and biochemical response should provide an excellent model system for the study of the regulation, function, and interrelationships of the various adrenergic receptors. Circumstances that may modify the balance between alpha-2 and beta receptors, e.g., fasting (39) and hypothyroidism (12, 40) should be especially interesting experimental paradigms.

ACKNOWLEDGMENTS

This work was supported by U. S. Public Health Service Research grants AM 10149, AM 11265, HL 16037, and HL 20399, as well as grant BNS 78 24715 from the National Science Foundation.

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