MOLECULAR PHARMACOLOGY, 25:64–69

Alpha$_1$-Adrenergic Stimulation of Phosphatidylinositol Turnover and Respiration of Brown Fat Cells

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Received March 11, 1983; Accepted August 24, 1983

SUMMARY

The *alpha*-adrenergic agonist phenylephrine (in the presence of the *beta*-adrenergic antagonist alprenolol) stimulated respiration and incorporation of [^{3}H]glycerol and [^{32}P] P_i into phosphatidylinositol of hamster brown fat cells in a concentration-dependent manner. Both responses were preferentially inhibited by prazosin as compared with yohimbine, indicating *alpha*$_1$ specificity. Uniquely, prazosin inhibition of phenylephrine-stimulated phosphatidylinositol metabolism had two components, since 30% of the response was inhibited by less than 1 nM prazosin, 10 nM gave no further inhibition, and 100 nM prazosin completely inhibited the response. The phosphatidylinositol response was still present in Ca^{2+}-free buffer, although reduced in magnitude. The concentration relationships of the effects of agonists and antagonists were compared with those of previous results of [^{3}H]prazosin binding and with phenylephrine potency to compete for binding. On the basis of these comparisons, it is suggested that the highly prazosin-sensitive part of the phosphatidylinositol response may be closely associated with receptor occupation.

INTRODUCTION

The role of *beta*-adrenoceptors in norepinephrine action on brown adipose tissue oxygen consumption (thermogenesis) is well documented (1). During exposure to cold, norepinephrine, released from sympathetic nerve terminals, activates hormone-sensitive lipase via cyclic AMP (1–3). The free fatty acids produced provide the mitochondria with substrate and may also play an important role as uncouplers of oxidative phosphorylation (3, 4) through the so-called "thermogenin" or "GDP-binding protein" which is a unique feature of brown adipocyte mitochondria (5, 6).

The presence of *alpha*-adrenergic receptors in rat brown adipose tissue has been inferred from effects on membrane depolarization (7–9) and [^{32}P]P_i uptake into phosphatidylinositol (10). Recently, Mohell *et al.* (11) demonstrated that hamster brown fat cells possess a large number of *alpha*$_1$-adrenergic binding sites which can be labeled with [^{3}H]prazosin. These binding sites appear to be connected to a physiological response, since approximately 20% of the norepinephrine-stimulated oxygen consumption originates from *alpha*$_1$-adrenergic pathways (12, 13).

In the present experiments we sought to extend the pharmacological description of *alpha*$_1$-adrenergic receptors in hamster brown fat cells by examining the "phosphatidylinositol response," which is a characteristic feature of this class of receptors. Furthermore, we examined the concentration relationships between phenylephrine regulation of phospholipid labeling and oxygen consumption as well as the effects of prazosin on these responses.

MATERIALS AND METHODS

Isolation of brown fat cells. Brown fat cells were isolated by collagenase digestion as first described for rat interscapular brown fat (14), with minor modifications found beneficial for hamster brown fat cells (15). Briefly stated, the interscapular, cervical, and axillary brown adipose tissue from male adult hamsters (*Mesocricetus auratus*), housed at 22° ± 2° and fed ad libitum, was removed, cleansed of contaminating tissues, and minced with scissors. The tissue pieces were digested in Krebs-Ringer phosphate buffer containing collagenase (2 mg/ml) at 37° for 10 min. After this preincubation, which resulted in cleaner cell preparations (15), the cell suspension was filtered through nylon chiffon, and the remaining tissue pieces were incubated for 30–45 min. The cells were filtered through nylon chiffon and washed three times by flotation. The cell suspension was diluted to contain approximately 10^6 cells/ml, counted in a Bürker chamber, and used for experiments the same day.

Measurement of incorporation of radioactivity into phospholipids. Isolated brown fat cells (approximately 300,000 cells) were incubated in 3 ml of Krebs-Ringer bicarbonate buffer with 10 μCi/ml of [^{3}H]

This research was supported by United States Public Health Service Research Grants AM 10149 and AM 21470 from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

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0026-895X/84/010064-06$02.00/0

TABLE 1

Effect of phenylephrine on incorporation of [^{3}H]glycerol and [^{32}P]P_i into phospholipids

Isolated brown fat cells (300,000–500,000 cells) were incubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 1 μM alprenolol, 10 μCi/ml of [^{3}H]glycerol, and [^{32}P]P_i, plus the indicated additions. The phospholipids were separated by 2-dimensional thin-layer chromatography as described under Materials and Methods. The results are expressed as means ± standard error of four experiments.

Phospholipid	Control	Phenylephrine, 10 μM	Phenylephrine, 10 μM + prazosin, 1 μM
	cpm/10^6 cells	*% of control*	*% of control*
[^{3}H]Glycerol incorporation			
Phosphatidic acid	2520 ± 810	198 ± 45	97 ± 6
Phosphatidylinositol	7290 ± 1420	167 ± 7	118 ± 12
Phosphatidylethanolamine	14450 ± 3150	96 ± 4	91 ± 5
Phosphatidylcholine	8540 ± 1850	100 ± 15	84 ± 8
[^{32}P]P_i incorporation			
Phosphatidic acid	620 ± 140	195 ± 42	89 ± 6
Phosphatidylinositol	950 ± 230	236 ± 48	89 ± 9
Phosphatidylethanolamine	860 ± 250	98 ± 6	84 ± 8
Phosphatidylcholine	1810 ± 570	89 ± 11	96 ± 11

glycerol and [^{32}P]P_i at 37° for 60 min under an atmosphere of 95% O_2 and 5% CO_2. The incubations were terminated by adding 0.25 ml of 80% trichloroacetic acid. The resulting precipitate was pelleted by centrifugation at 1500 × *g* for 5 min, after which the supernatant was removed and the pellet was extracted into 3 ml of $CHCl_3$/MeOH (2:1, v/v). The sample tubes were capped and shaken at room temperature for 30 min. Then, 1.5 ml of 2 M KCl were added and the extracts were vortexed and centrifuged at 1500 × *g* for 7 min. The upper aqueous phase and the interphase were removed and the organic phase was sampled. The aliquots were evaporated to dryness *in vacuo*, and the lipids were redissolved in $CHCl_3$ for chromatography.

Two different chromatograhic systems were used to separate phospholipids. Separations were performed in one dimension using $CHCl_3$/MeOH/28% NH_3OH/H_2O (130:70:6:4) as solvents and silica gel H thin-layer plates, supplemented with 3% magnesium acetate. This system adequately separates phosphatidylinositol from other phospho- and neutral lipids. Separations were performed in two dimensions with silica gel GHL plates (Analtech, Inc.), first using the solvent petroleum ether/anhydrous ether/acetic acid (50:50:1), followed in the same dimension (after drying the plates briefly) by the solvent $CHCl_3$/MeOH/28% NH_3OH/H_2O (130:70:6:4). Separation in the second dimension was accomplished with 1-butanol/acetic acid/H_2O (6:1:1) as solvents as described by Abdel-Latif *et al.* (16).

Phospholipids were visualized on chromatography plates by exposure to I_2 vapors. Areas containing individual phospholipids were scraped into scintillation vials and the radioactivity was determined using 0.25 ml of water and 5 ml of Biofluor (New England Nuclear Corporation). Phosphorus determinations were performed as described by Bartlett (17). All data are based on duplicate samples for various conditions in each experiment.

Measurements of oxygen consumption. Isolated adipocytes (approximately 250,000 cells) were added to siliconized Warburg flasks containing 2 ml of Krebs-Ringer phosphate buffer, and the oxygen consumption was measured in a Gilson respirometer at 37° for 60 min as earlier described (3).

Buffers. Krebs-Ringer phosphate buffer had the following composition (millimolar): 148 Na^+, 6.9 K^+, 1.5 Ca^{2+}, 1.4 Mg^{2+}, 120 Cl^-, 1.4 SO_4^{2-}, 5.2 $H_2PO_4^-$, 16.8 HPO_4^{2-}, 10 glucose, 10 fructose, and 4% bovine serum albumin (pH 7.4).

Krebs-Ringer bicarbonate buffer had the following composition (millimolar): 145 Na^+, 6.0 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 130 Cl^-, 1.2 SO_4^{2-}, 25.3 HCO_3^-, 1.2 HPO_4^{2-}, 10 glucose, 10 fructose, and 4% bovine serum albumin (pH 7.4).

Chemicals. Chemicals were obtained from the following sources: norepinephrine, phenylephrine, yohimbine, and alprenolol from Sigma Chemical Company; prazosin from Pfizer; [^{3}H]glycerol (200 mCi/mmole) and [^{32}P]P_i (carrier-free) from New England Nuclear Corporation; bovine serum albumin (Fraction V) from Miles Laboratories; and collagenase from Boehringer Mannheim Biochemicals. Other reagents were analytical grade, of the best quality available.

RESULTS

Incorporation of [^{3}H]glycerol and [^{32}P]P_i into phospholipids: effect of phenylephrine and prazosin. Phenylephrine addition to hamster brown adipocytes selectively increased the incorporation of both [^{32}P]P_i and [^{3}H]glycerol into phosphatidylinositol and phosphatidic acid, whereas the labeling of phosphatidylethanolamine and phosphatidylcholine was unaffected (Table 1). The effect of phenylephrine on uptake of label was not accompanied by detectable increases in phospholipid content (Table 2).

Unlike white adipose tissue, rat brown adipose tissue contains considerable glycerokinase activity (18). These results demonstrate that hamster brown adipocytes contain glycerokinase as well. Uptake of [^{3}H]glycerol into phospholipids can thus be used as an index of *de novo* synthesis. [^{32}P]P_i incorporation into phospholipids can result either through *de novo* synthesis or, as common

TABLE 2

Phospholipid composition of hamster brown fat cells: effect of phenylephrine

Isolated brown fat cells (300,000–500,000 cells) were incubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 1 μM alprenolol plus the indicated additions. The phospholipids were separated by 2-dimensional thin-layer chromatography, and each spot was analyzed for total phosphorus. Approximately 78 μg of lipid phosphorus was recovered per 10^6 cells. The data shown are fractions of total lipid phosphorus present in each phospholipid. The values are means ± standard error of three experiments.

Phospholipid	Control	+ Phenylephrine 10 μM
	% of total lipid phosphorus	
Phosphatidic acid	1.3 ± 0.3	1.3 ± 0.2
Phosphatidylinositol	2.3 ± 0.2	2.7 ± 0.7
Phosphatidylethanolamine	17.6 ± 1.1	16.5 ± 1.0
Phosphatidylcholine	43.9 ± 0.8	43.9 ± 2.4
Phosphatidylserine	2.7 ± 0.2	2.8 ± 0.3
Other phospholipids	32.1	32.6

for phosphatidylinositol, by resynthesis from its diacyl glycerol moiety. [^{3}H]Glycerol uptake into phospholipids cannot be measured if considerable lipolysis (due to *beta*-adrenergic effects) is occurring, because of pool dilution with unlabeled glycerol. However, no lipolysis was seen in brown fat cells incubated with as much as 50 μM phenylephrine in the presence of 1 μM alprenolol (data not shown). This is due to the relative specificity of phenylephrine for *alpha*-receptors.

In isolated white fat cells, high concentrations of propranolol stimulated the incorporation of [^{32}P]P_i into phosphatidylinositol (19). In our experiments, 1 μM alprenolol did not have any effect on phospholipid turnover. In the presence of 1 μM alprenolol, [^{3}H]glycerol uptake was 105 ± 9% and [^{32}P]P_i uptake was 104 ± 9% (mean ± standard error of nine experiments) as compared with control cells.

The [^{32}P]P_i labeling pattern and the effects of phenylephrine are in good agreement with the increases seen in rat brown adipocytes after incubation with epinephrine (10) or in hamster brown adipose tissue after the injection of norepinephrine *in vivo* (20). In agreement with the suggestion (21) that the effects of catecholamines on turnover of phosphatidylinositol and phosphatidic acid are mediated through *alpha*$_1$-receptors, the effect of 10 μM phenylephrine was abolished by 1 μM prazosin (a specific *alpha*$_1$-adrenergic antagonist) (Table 1). Prazosin by itself was without effect.

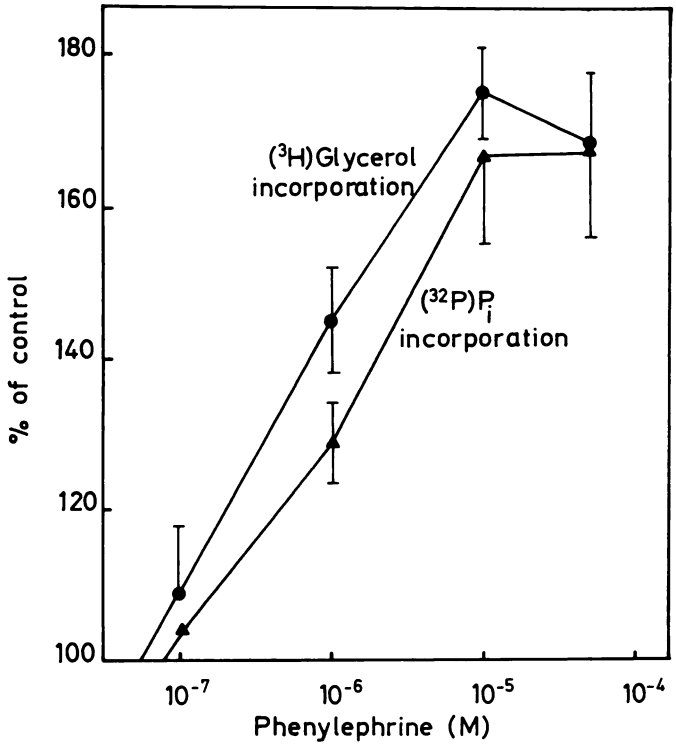

FIG. 1. *Dose-response curves for phenylephrine-stimulated incorporation of [^{3}H]glycerol and [^{32}P]P_i into phosphatidylinositol*

Brown fat cells (200,000–500,000 cells) were incubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 10 μCi/ml of [^{3}H] glycerol and [^{32}P]P_i, 1 μM alprenolol, and different concentrations of phenylephrine. The values are means ± standard error of five duplicate determinations of as many different cell preparations and are expressed as percentage of the values in the presence of 1 μM alprenolol. The basal uptake of [^{3}H]glycerol (●) into phosphatidylinositol was 9450 cpm/10^6 cells and for [^{32}P]P_i (▲) into phosphatidylinositol it was 2020 cpm/10^6 cells.

Concentration-response curves for phenylephrine-stimulated incorporation of [^{3}H]glycerol and [^{32}P]P_i into phosphatidylinositol. Incubation of isolated cells in the presence of 10 μCi/ml of [^{3}H]glycerol and [^{32}P]P_i, 1 μM alprenolol and different concentrations of phenylephrine increased the labeling of phosphatidylinositol as shown in Fig. 1. The effect of phenylephrine was dose-dependent, and the EC_{50} values for [^{3}H]glycerol and [^{32}P]P_i uptake were 0.6 μM and 1.3 μM, respectively. The percentage increases with 10 μM phenylephrine in 16 experiments were 78 ± 9% for [^{3}H]glycerol incorporation and 90 ± 12% for [^{32}P]P_i incorporation (mean ± standard error).

Effect of alpha-adrenergic antagonists on phosphatidylinositol turnover. Concentration-response curves for antagonist inhibition of phosphatidylinositol labeling are shown in Figs. 2 and 3. When brown fat cells were incubated in the presence of 10 μM phenylephrine, 1 μM alprenolol, and increasing concentrations of prazosin, biphasic inhibition of incorporation of [^{3}H]glycerol and [^{32}P]P_i into phosphatidylinositol occurred. While 0.1 nM prazosin inhibited by 30% the phenylephrine-stimulated [^{3}H]glycerol incorporation into phosphatidylinositol, increasing the concentration to 10 nM gave little further inhibition (Fig. 2). However, 100 nM prazosin totally blocked the stimulation due to 10 μM phenylephrine (Fig. 2). Inhibition of [^{32}P]P_i incorporation by prazosin was also biphasic, although somewhat less pronounced. The concentration-response curve for yohimbine inhibition of [^{3}H]glycerol incorporation was shifted to the right (Fig. 3). Yohimbine was approximately 100 times less potent than prazosin. Quantitatively and qualitatively similar results were obtained for yohimbine inhibition of [^{32}P]P_i labeling (data not shown).

Effect of extracellular Ca^{2+}. Hormonal activation of phosphatidylinositol turnover by *alpha*$_1$-adrenoceptors is thought to be linked in some way to Ca^{2+} mobilization (22). To test the Ca^{2+} dependence of hormone-stimulated phosphatidylinositol labeling, brown adipocytes were incubated in Krebs-Ringer bicarbonate buffer (2.5 mM Ca^{2+}) with or without 10 μM phenylephrine in the presence or absence of 3.5 mM EGTA.[3] Incubation with EGTA slightly reduced the basal uptake of label into phosphatidylinositol while the phenylephrine-stimulated uptake was reduced by about 50% (Table 3). However, part of the phenylephrine effect was independent of extracellular Ca^{2+}. Schimmel *et al.* (13) observed that the stimulation of hamster brown adipocyte respiration by phenylephrine was abolished by adding EGTA (3.0 mM) to buffer containing 2 mM Ca^{2+}.

Phenylephrine-stimulated respiration of brown fat cells. Phenylephrine in the presence of 1 μM alprenolol stimulated the oxygen consumption of brown fat cells in a concentration-dependent manner with an EC_{50} value of 100 nM (Fig. 4). Basal respiration was approximately 110 nmoles of oxygen per 10^6 cells per minute. The maximal

[3] The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

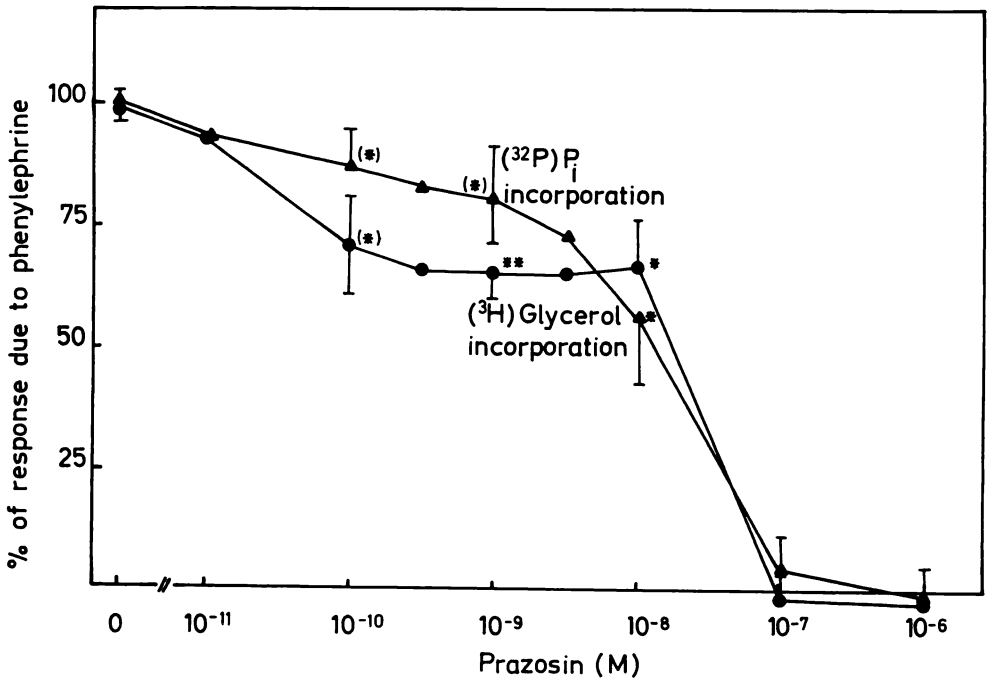

FIG. 2. *Inhibition of phenylephrine-stimulated incorporation of [^{3}H]glycerol and [^{32}P]P_i into phosphatidylinositol by prazosin*

Isolated hamster brown fat cells (300,000–500,000 cells) were incubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 10 μCi/ml of [^{3}H]glycerol and [^{32}P]P_i, 1 μM alprenolol, 10 μM phenylephrine, and the indicated concentrations of prazosin. The increment due to phenylephrine over basal values was taken as 100%. The control values for the incorporation of [^{3}H]glycerol and [^{32}P]P_i into phosphatidylinositol were 11,600 ± 2,900 and 1,920 ± 310 cpm/10^6 cells, respectively. The percentage increases with 10 μM phenylephrine were 88 ± 14% for [^{3}H] glycerol incorporation (●) and 76 ± 17% for [^{32}P]P_i incorporation (▲). Points with standard error indicated are means of four to six duplicate determinations of as many different cell preparations. (*), *, and ** denote significant effects of prazosin ($p < 0.1$, 0.05, and 0.01, respectively; Student's paired t-test, n = 4–6). Points without standard error indicated are means of two duplicate determinations of two different cell preparations.

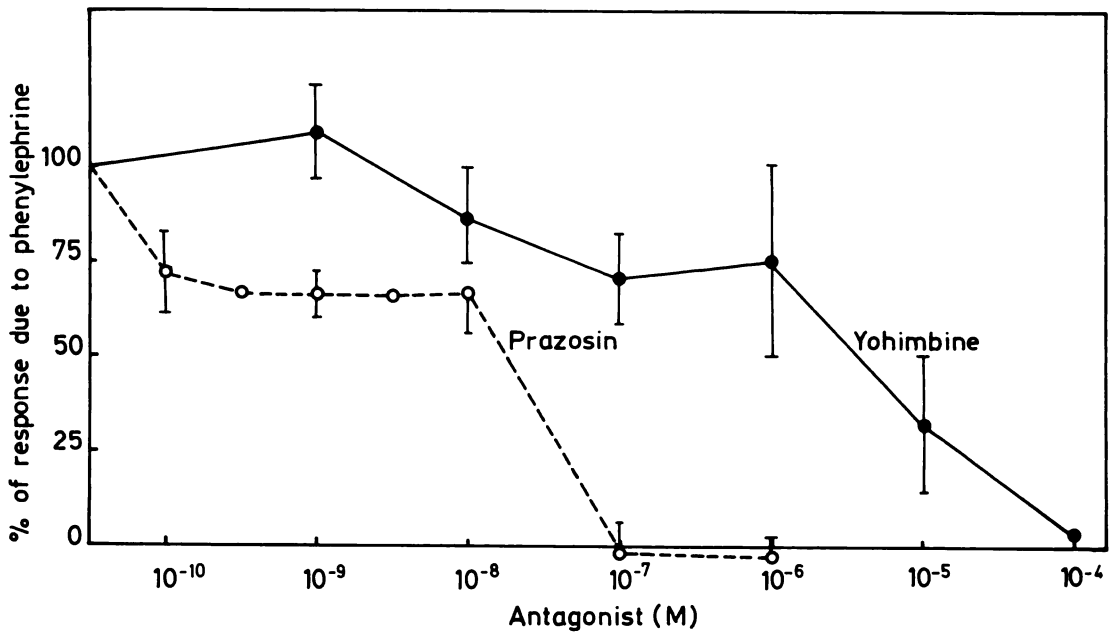

FIG. 3. *Inhibition of phenylephrine-stimulated incorporation of [^{3}H]glycerol into phosphatidylinositol by yohimbine as compared with prazosin*

Isolated brown fat cells (300,000–500,000 cells) were incubated for 60 min in the presence of 10 μCi/ml of [^{3}H]glycerol , 1 μM alprenolol, 10 μM phenylephrine, and the indicated concentrations of yohimbine (●) or prazosin (○). The values are expressed as percentages of the stimulation due to 10 μM phenylephrine alone and are means ± standard error of five duplicate determinations of as many different cell preparations.

TABLE 3

Effect of Ca^{2+} on the incorporation of radioactivity into phosphatidylinositol (PI)

Brown fat cells (300,000–500,000 cells) were incubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 2.5 mM Ca^{2+}, 10 μCi of [^{3}H]glycerol, and [^{32}P]P_i plus 1 μM alprenolol with or without 3.5 mM EGTA. The results are means ± standard error of six experiments.

Addition	Control	+ Phenylephrine, 10 μM
	cpm/10^6 cells	*% of control*
[^{3}H]Glycerol uptake into PI		
Without	6230 ± 2030	213 ± 22
+ 3.5 mM EGTA	5050 ± 250	153 ± 10
[^{32}P]P_i uptake into PI		
Without	1215 ± 245	201 ± 14
+ 3.5 mM EGTA	1020 ± 40	167 ± 10

respiration with phenylephrine was 175 ± 20 nmoles of oxygen per 10^6 cells per minute, which was approximately 30% of the increase seen with 2 μM norepinephrine, tested as a control of every cell preparation ($n = 5$). The phenylephrine-stimulated increase in oxygen consumption was inhibited by 100 nM prazosin, whereas 100 nM yohimbine slightly potentiated the effect of the agonist (data not shown).

DISCUSSION

The present results delineate an *alpha*-adrenoceptor-mediated stimulation of *de novo* phosphatidylinositol and phosphatidic acid synthesis in isolated hamster brown fat cells, as has been found in many other cells (22). Phenylephrine (in the presence of 1 μM alprenolol) stimulated the incorporation of both [^{3}H]glycerol and [^{32}P]P_i into phosphatidylinositol in a concentration-dependent manner with an EC_{50} value of about 1 μM (Fig. 1). This response was inhibited by low concentrations of prazosin, demonstrating *alpha*$_1$ specificity. This hormone-stimulated synthesis of phosphatidylinositol in brown fat cells primarily represents *de novo* synthesis of the molecule, since [^{3}H]glycerol was incorporated into phosphatidylinositol to the same extent as was [^{32}P]P_i.

The results with brown adipocytes demonstrate a biphasic inhibition curve of the phosphatidylinositol response by the *alpha*$_1$-adrenergic antagonist, prazosin (Fig. 2). The first part (i.e., 20–30% of maximal phosphatidylinositol labeling) was inhibited by prazosin in concentrations between 0.1 and 10 nM, and the second (i.e., the last 70–80% of maximal labeling) was blocked by 100 nM prazosin. The underlying mechanisms involved in these biphasic inhibition curves are not known. Possibly the phosphatidylinositol labeling that is inhibited by low prazosin concentrations reflects events associated with phosphatidylinositol hydrolysis (and compensatory resynthesis) directly linked to hormone-receptor interactions in the plasma membrane. This is supported by the fact that the apparent IC_{50} value of the first part of the prazosin inhibition curve was less than 1 nM (Fig. 2) and is thus comparable to the K_D value of (^{3}H)prazosin binding (0.4 nM) to *alpha*$_1$-receptors of these cells (Table 5). Further indication may be obtained from the fact that the phenylephrine-stimulated phosphatidylinositol response had an approximately 10-fold lower sensitivity to hormone than the phenylephrine-stimulated oxygen consumption measured in these cells (Table 4). Thus, at least part of the phosphatidylinositol response seems to be more "closely" linked to hormone-

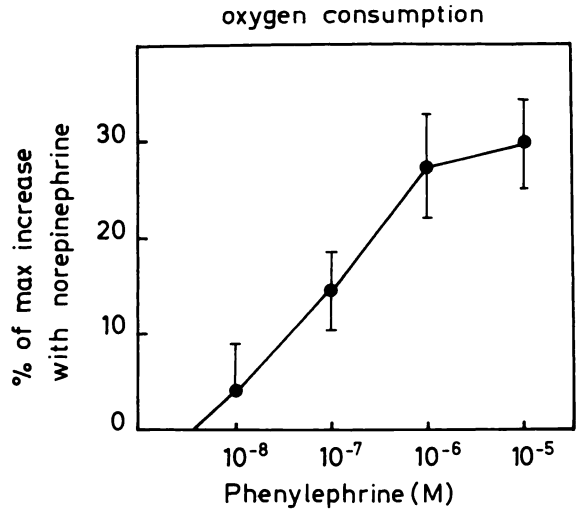

FIG. 4. *Phenylephrine-stimulated oxygen consumption of brown fat cells*

Brown fat cells (about 250,000 cells) were incubated in 2 ml of Krebs-Ringer phosphate buffer with 1 μM alprenolol and the indicated concentrations of phenylephrine as described under Materials and Methods. The increase in respiration is expressed as percentage of the increase seen with 2 μM norepinephrine. The results are means ± standard error of five triplicate determinations of as many different cell preparations.

TABLE 4

Comparison of EC_{50} values for agonist stimulation of oxygen consumption, for incorporation of radioactivity into phosphatidylinositol (PI), and K_i for agonist inhibition of [^{3}H]prazosin binding

K_i values were determined according to the equation $K_i = IC_{50}/(1 + (L)/K_D)$ (23).

	Source	EC_{50}	K_i	Potency ratio
		nM	*nM*	
Alpha$_1$ component of norepinephrine-stimulated oxygen consumption	Ref. 12	50		1
Phenylephrine-stimulated oxygen consumption	Fig. 4	100		0.5
Phenylephrine-stimulated uptake of [^{3}H] glycerol into PI	Fig. 1	600		0.08
Phenylephrine-stimulated uptake of [^{32}P]P_i into PI	Fig. 1	1300		0.04
Norepinephrine displacement of [^{3}H]prazosin binding	Ref. 11		330	0.15
Phenylephrine displacement of [^{3}H]prazosin binding	Ref. 11		260	0.19

TABLE 5

Comparison of K_i values for antagonist inhibition of oxygen consumption, phosphatidylinositol (PI) response, and [^{3}H]prazosin binding

K_i values were determined as in Table 4.

	Source	Prazosin K_i	Yohimbine K_i
		nM	*nM*
Inhibition of [^{3}H]prazosin binding	Ref. 11	0.37	390
Inhibition of *alpha*$_1$ component of norepinephrine-stimulated oxygen consumption	Ref. 12	3.5	1800
Inhibition of phenylephrine-stimulated incorporation of [^{3}H]glycerol into PI	Fig. 3		
First part of the antagonist inhibition curve[a]		0.1	10
Second part of the antagonist inhibition curve[a]		10	1000

[a] These K_i values are estimates, since the actual K_D values for phenylephrine for the two components of the PI response are not known.

receptor occupation than the respiration response, which may be mediated via a second messenger (12).

Mohell *et al.* (11) found that brown fat cells exhibit a "coupling ratio" of about 5–10 between *alpha*$_1$-receptor occupation and *alpha*$_1$-receptor mediated respiration; i.e., only 10–20% of receptors have to be occupied by agonist in order to achieve full physiological response, and, vice versa, 80–90% of receptors have to be occupied by antagonist in order to see any inhibition of agonist-stimulated respiration. Although the first part of the phosphatidylinositol response was inhibited by very low prazosin concentrations, the second part of the prazosin inhibition curve had an apparent IC_{50} value comparable to prazosin inhibition of oxygen consumption (Table 5).

The concentration relationships of agonist and antagonist effects for different *alpha*$_1$-adrenergic responses of hamster brown fat cells are summarized in Tables 4 and 5. On the basis of these comparisons, it is suggested that brown fat cells possess both a "primary" (possibly Ca^{2+}-independent) and a "secondary" (possibly Ca^{2+}-dependent) phosphatidylinositol response. Respiration due to *alpha*$_1$ stimulation appears to be a secondary Ca^{2+}-dependent response (13), but only part of the increase in phosphatidylinositol synthesis was abolished in Ca^{2+}-free buffer. These data indicate that increased turnover of phosphatidylinositol can be seen under conditions wherein brown adipocytes are so Ca^{2+}-depleted that respiration cannot be stimulated. In some tissues the initial hormone-stimulated breakdown of phosphatidylinositol in the plasma membrane seems to release trigger Ca^{2+}, which also influences phosphatidylinositol turnover (for review see ref. 22). Whether the two different phosphatidylinositol responses in isolated brown fat cells are sequential or possibly mediated via different *alpha*$_1$-receptor subpopulations remains to be shown.

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