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β-Adrenergic Receptors in Rat Fat Cells and Their Relationship with Lipolysis

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Norepinephrine stimulated lipolysis in rat fat cells while (−)-alprenolol completely inhibited this lipolysis. (−)-Alprenolol competed for (−)-[^{3}H]dihydroalprenolol(DHA) binding sites on rat fat cells. The specific (−)-[^{3}H]DHA binding sites identified by competition with (−)-alprenolol were found to be transferred to the solubilized supernatant during preparation of endogenous lipid droplets from the fat cells. Although the lipid droplets did not exhibit specific (−)-[^{3}H]DHA binding, norepinephrine induced lipolysis in a cell-free system consisting of the lipid droplets and hormone-sensitive lipase (HSL). Norepinephrine-induced lipolysis in the cell-free system was inhibited by propranolol and (−)-alprenolol, but not by phenoxybenzamine. The lipolytic action of norepinephrine and the anti-lipolytic actions of propranolol and (−)-alprenolol disappeared after sonication of the lipid droplets in the cell-free system. These results suggest that the adrenergic receptor concerned with lipolysis in fat cells may not be a specific (−)-[^{3}H]DHA binding site, but may be closely related to the lipid droplets.

Key words: fat cell, lipid droplet, lipolysis, specific binding.

It is well known that catecholamines play a central role in promoting lipolysis by white fat cells. This effect is believed to be mediated by a β-adrenergic receptor-adenylate cyclase complex, which is located in the plasma membrane of fat cells and consists of at least three distinct components, these being a β-adrenergic receptor, a nucleotide regulatory protein and adenylate cyclase (*1*). The receptor-controlled increase in intracellular cyclic AMP concentrations promotes activation of cyclic AMP-dependent protein kinase A, which phosphorylates a serine residue (Ser-563 for the rat and Ser-551 for the human) on HSL and promotes its activation. The resulting phosphorylated HSL is believed to stimulate lipolysis in fat cells. The theory of catecholamine-induced lipolysis is widely accepted, though we have reported experimental results which are in conflict with this theory (*2*).

Previously, we suggested that the phosphorylated HSL might not stimulate lipolysis in fat cells. Cyclic AMP-dependent activation of HSL certainly stimulated lipolysis of artificial lipid droplets such as [^{3}H]triolein emulsified with gum arabic, whereas it failed to stimulate that of endogenous lipid droplets from rat fat cells. The endogenous lipid droplets were prepared from the fat cells by mild procedures, including hypotonic shock, Triton X-100 treatment, and brief incubation (37°C, 10 min) in a buffer. Approximately 95% of the protein present in the cells was removed from the droplets which consisted of (per gram dry weight) 870 μmol of triglyceride, 0.53 μmol of phosphatidylcholine, 0.18 μmol of phosphatidylethanolamine, 0.52 μmol of cholesterol, 342 μg of carbohydrate, and 63 μg of protein. Addition of epinephrine to the droplets did not stimulate lipolysis in the absence of added lipase, indicating that the droplets did not contain fat cells (*3, 4*). It was suggested that loss of responsiveness of the droplets in fat cells to activated HSL might be due to phosphatidylcholine on the surface of the droplets (*2*).

The fact that cyclic AMP-dependent activation of HSL did not stimulate lipolysis of the endogenous lipid droplets, suggests that catecholamine-induced lipolysis in fat cells may not be mediated by β-adrenergic receptor-adenylate cyclase-protein kinase A-HSL cascade. Therefore, the present study was designed to clarify whether or not β-adrenergic receptors participate in catecholamine-induced lipolysis in rat fat cells.

MATERIALS AND METHODS

Animals—Young male Wistar-King rats, weighing 150 to 200 g, were given a standard laboratory diet and water *ad libitum*. They were killed by cervical dislocation to minimize endogenous catecholamine secretion, and their epididymal adipose tissues were quickly removed.

Materials—Collagenase (type IV) was purchased from Worthington Biochemical (Freehold, NJ). *N*-Tris(hydroxymethyl)methyl-1-2-aminoethanesulfonic acid (TES) was obtained from Wako Pure Chemical (Osaka). (±)-Norepinephrine was purchased from Sankyo (Tokyo). (±)-Propranolol hydrochloride and phenoxybenzamine hydrochloride were supplied by Nacalai Tesque (Kyoto). (−)-Alprenolol was purchased from Sigma (St. Louis, MO). (−)-[^{3}H]DHA (58.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Bovine serum albumin containing 1.22 μmol

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Abbreviations: HSL, hormone-sensitive lipase; TES, *N*-tris(hydroxymethyl)methyl-1-2-aminoethanesulfonic acid; (−)-[^{3}H]DHA, (−)-[^{3}H]dihydroalprenolol.

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of free fatty acids per gram was obtained from Wako Pure Chemical Industries and extracted to remove the free fatty acids by the method of Chen (5).

Measurement of Lipolytic Activity in Fat Cells—Isolated fat cells were obtained from rat epididymal adipose tissues by the method of Rodbell (6). The resultant fat cell fraction (50 μl packed volume) was incubated for 1 h at 37°C in 225 μl of buffer A (25 mM TES, pH 7.4, containing 135 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$) supplemented with 2.5% bovine serum albumin and 25 μl of (±)-norepinephrine solution in buffer A. The free fatty acids released were measured as described previously (7). Briefly, the incubation mixture (250 μl) was mixed with 3 ml of a 1 : 1 (v/v) mixture of chloroform and heptane containing 2% (v/v) methanol and extracted by shaking the tube horizontally for 10 min in a shaker. The mixture was centrifuged (2,000 × *g*, 5 min), the upper aqueous phase was removed by suction, and 1 ml of copper reagent was added to the lower organic phase. The tube was shaken for 10 min, the mixture was centrifuged (2,000 × *g*, 10 min), and 0.5 ml of the upper organic phase, which contained the copper salts of the extracted fatty acids, was treated with 0.5 ml of 0.1% (w/v) bathocuproin in chloroform containing 0.05% (w/v) 3-*tert*-butyl-4-hydroxyanisole. Its absorbance at 480 nm was then measured. Lipolysis was expressed as microequivalents (μEq) of free fatty acids (FFA) released per ml of packed fat cells per h.

β-Adrenergic Receptor Assay—β-Adrenergic receptor assay was carried out using (−)-[^{3}H]DHA according to the procedure of Cabelli and Malbon (8). Packed fat cells (50 μl) were incubated with 10 nM (−)-[^{3}H]DHA for 10 min at 37°C in a total volume of 1 ml of buffer A containing 0.2% albumin with constant, low-speed shaking. At the end of the incubation, 6 ml of ice-cold wash buffer (buffer A at 4°C) was added to each tube (Falcon 17 × 100 mm, No. 2018 tubes) to terminate the reaction. The contents of the tube were rapidly filtered under vacuum through a single Whatman GF/C filter (24 mm in diameter) that had been prewashed with 6 ml of buffer. The filters were then washed with two 6 ml aliquots of ice-cold wash buffer, placed directly into glass scintillation vials, and counted. Control tubes containing no fat cells were included in all experiments. Nonspecific binding was determined by assaying bound radioligand in the presence of 10^{-4} M (−)-alprenolol.

Preparation of Endogenous Lipid Droplets—Isolated fat cells were obtained by the method of Rodbell (6), and 1 ml (packed volume) of cells was suspended in 4 ml of 5 mM Tris-HCl buffer (pH 7.4). The suspension was mixed by slowly inverting the centrifuge tube three times and then centrifuged at 200 × *g* for 3 min at room temperature. The fat layer was mixed with 4 ml of 5 mM Tris-HCl buffer (pH 7.4) containing 0.025% Triton X-100 by slowly inverting the tube three times and the mixture was centrifuged at 200 × *g* for 3 min at room temperature. The fat layer was washed once with buffer A, incubated with buffer A at 37°C for 10 min, centrifuged at 200 × *g* for 3 min, and washed again with buffer A. The resulting fat layer was used as endogenous lipid droplets in the experiments.

Preparation of Lipoprotein Lipase-Free HSL Solution—Rat epididymal adipose tissue (0.7 g) was cut into small pieces with scissors and hand-homogenized in 1 ml of buffer A in a Potter-Elvehjem homogenizer by five strokes of a Teflon pestle. The homogenate was centrifuged (2,500 × *g*, 15 min) at 10°C, resulting in precipitation of plasma membrane. The supernatant was applied to a heparin-Sepharose column (5 × 20 mm), equilibrated with buffer A, to remove lipoprotein lipase. The unadsorbed fraction was used as the HSL solution. HSL activity in this fraction was not reduced by 1 M NaCl or antiserum to bovine lipoprotein lipase, indicating that lipolytic activity due to lipoprotein lipase was minimal in the preparation.

Lipolysis in a Cell-Free System Consisting of HSL and Endogenous Lipid Droplets—A sample of 25 μl of packed endogenous lipid droplets was incubated at 37°C for 1 h with 100 μl of HSL solution, 25 μl of (±)-norepinephrine solution, 100 μl of buffer A containing 2.5% bovine serum albumin, and 25 μl of buffer A. Another sample of 375 μl of packed endogenous lipid droplets was mixed with 750 μl of buffer A containing 2.5% bovine serum albumin and 16.95 mg of gum arabic. The mixture was sonicated for 5 min, and the sonicate (75 μl) was incubated with 100 μl of HSL solution and 100 μl of buffer A containing 2.5% bovine serum albumin for 1 h at 37°C. Lipolysis was expressed as μEq FFA per ml of packed lipid droplets per h.

Analysis of Data—Student's *t* test was used to determine the significance of differences.

RESULTS

As shown in Fig. 1, norepinephrine stimulated lipolysis in fat cells at concentrations over 10^{-7} M and maximal lipolysis occurred at concentrations over 10^{-6} M. (−)-Alprenolol slightly reduced norepinephrine-induced lipolysis at 10^{-7} M and completely inhibited it at concentrations over 10^{-5} M. Cabelli and Malbon first reported a method to identify β-adrenergic receptors in isolated rat fat cells with tritium-labeled (−)-alprenolol or (−)-[^{3}H]DHA (8). As (−)-alprenolol completely inhibited the hormone-induced lipolysis, we tried to examine specific (−)-[^{3}H]DHA binding sites, or β-adrenergic receptors, according to their procedure.

As shown in Fig. 2, total (−)-[^{3}H]DHA binding to fat cells was reduced 32% by 10^{-4} M (−)-alprenolol. Specific

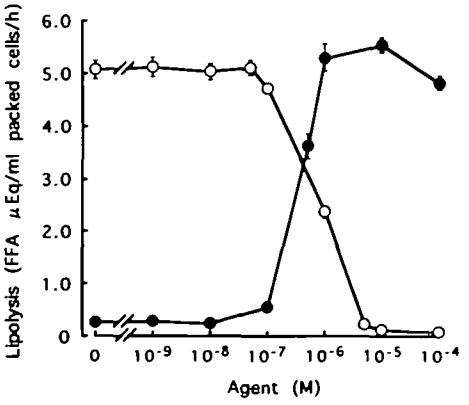

Fig. 1. **Norepinephrine-induced lipolysis and (−)-alprenolol-mediated inhibition of lipolysis in fat cells.** The procedure was as described in "MATERIALS AND METHODS." Lipolysis in the presence of (±)-norepinephrine is indicated by (●) and that with 6 μM (±)-norepinephrine and (−)-alprenolol by (○). Each point represents the mean ± SE of four separate experiments.

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(−)-[^{3}H]DHA binding to fat cells was 0.44 pmol/g packed fat cells (Table I).

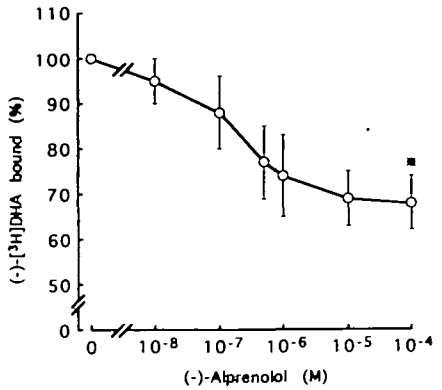

Fig. 2. **Inhibition of (−)-[^{3}H]dihydroalprenolol binding to fat cells by (−)-alprenolol.** The procedure was as described in "MATERIALS AND METHODS." Fat cells were incubated with 10 nM radioligand for 10 min at 37°C in the presence of (−)-alprenolol (○). Each point represents the mean±SE of four separate experiments. *$p<0.01$ *vs.* absence of (−)-alprenolol.

TABLE I. **Specific (−)-[^{3}H]dihydroalprenolol binding to fat cells and lipid droplets.** Specific binding to fat cells was examined with 10 nM (−)-[^{3}H]DHA in the presence or absence of 10^{-4} M (−)-alprenolol. Lipid droplets were prepared as described in "MATERIALS AND METHODS" from fat cells prelabeled with the radioligand in the presence or absence of 10^{-4} M (−)-alprenolol. The lipid droplets and the buffer solutions used in the preparation of lipid droplets were subjected to estimation of radioactivity after washing. Values are means±SE of four separate experiments.

Preparation	Binding 10^{-4} M (−)-Alprenolol		Specific binding	
	− (cpm)	+ (cpm)	(cpm/g)	(pmol/g)
Fat cells	2,833±215	1,923±174*	18,200	0.44
Lipid droplets	1,744±109	1,723±129$^{n.s.}$	—	—
Solubilized supernatant	621±24	237±25*	7,680	0.25

*$p<0.01$ *vs.* absence of 10^{-4} M (−)-alprenolol.

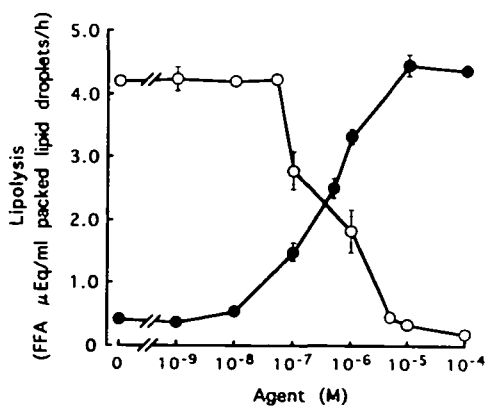

Fig. 3. **Norepinephrine-induced lipolysis and (−)-alprenolol-mediated inhibition of lipolysis in a cell-free system.** The procedure was as described in "MATERIALS AND METHODS." Lipolysis in the presence of (±)-norepinephrine is indicated by (●) and that with 6 μM (±)-norepinephrine and (−)-alprenolol by (○). Each point represents the mean±SE of four separate experiments.

After incubation of fat cells with 10 nM (−)-[^{3}H]DHA in the presence or absence of 10^{-4}M (−)-alprenolol, lipid droplets were prepared. About 60% of the specific (−)-[^{3}H]DHA binding component in the fat cells was found to be transferred to the solubilized supernatant during preparation of the lipid droplets.

As shown in Table I, the specific binding component was found in the solubilized supernatant, whereas the lipid droplets did not contain any specific binding component. Although the lipid droplets did not exhibit specific (−)-[^{3}H]DHA binding, norepinephrine induced lipolysis in a cell-free system consisting of the lipid droplets and plasma membrane-depleted HSL (Fig. 3). The maximal rate of lipolysis produced by norepinephrine was 5.53 μEq free fatty acid/ml of packed cells/h in fat cells (Fig. 1) and 4.46 μEq free fatty acid/ml packed lipid droplets/h in the cell-free system (Fig. 3). Triglyceride content in 1 ml of packed fat cells was 25 mg and that in 1 ml of packed lipid droplets was 28 mg. Therefore, 4.46 μEq of free fatty acid/ml of packed lipid droplets corresponded to 3.98 μEq of free fatty acid/ml of packed fat cells. (−)-Alprenolol inhibited norepinephrine-induced lipolysis in this cell-free system (Fig. 3). Sonication of the lipid droplets in the cell-free system induced almost the same rate of lipolysis in the absence of norepinephrine as the maximal rate induced

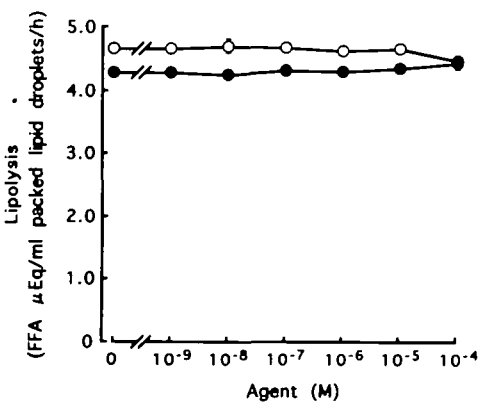

Fig. 4. **Effect of norepinephrine and (−)-alprenolol on lipolysis in a cell-free system containing sonicated instead of endogenous lipid droplets.** The procedure was as described in "MATERIALS AND METHODS." Lipolysis in the presence of (±)-norepinephrine is indicated by (●) and that with (−)-alprenolol and 6 μM (±)-norepinephrine by (○).

TABLE II. **Effects of α- and β-blockers on lipolysis in the cell-free system.** The procedure was as described in "MATERIALS AND METHODS." Values are means±SE of four separate experiments.

Addition	Lipolysis (FFA μEq/ml packed lipid droplets/h)	
	Intact lipid droplets	Sonicated lipid droplets
None	0.47±0.07	4.69±0.09
NE* (6 μM)	4.21±0.18	4.68±0.03
NE (6 μM)+ phenoxybenzamine (10^{-6} M)	4.19±0.26	4.79±0.13
NE (6 μM)+ (±)-propranolol (10^{-6} M)	2.31±0.14	4.69±0.05
NE (6 μM)+ (−)-alprenolol (10^{-6} M)	1.45±0.17	4.69±0.09

*NE, (±)-norepinephrine.

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by the hormone in intact lipid droplets (Fig. 4). The rate of lipolysis was not enhanced by further addition of norepinephrine and was not inhibited by addition of (−)-alprenolol. Norepinephrine stimulated lipolysis in the cell-free system containing intact lipid droplets and HSL. Hormone-induced lipolysis was not reduced by 10^{-6} M phenoxybenzamine (α-blocker) but was inhibited by 10^{-6} M propranolol (β-blocker) or by 10^{-6} M (−)-alprenolol (Table II). In contrast, a high lipolysis rate was found even in the absence of norepinephrine in the cell-free system consisting of sonicated lipid droplets and HSL. This rate was not changed by the addition of norepinephrine, phenoxybenzamine, propranolol and/or (−)-alprenolol (Table II).

These results indicate that intact lipid droplets contain norepinephrine, (±)-propranolol (β-blocker) and/or (−)-alprenolol (β-blocker)-sensitive, and phenoxybenzamine (α-blocker)-insensitive, receptors, and that sonication of the lipid droplets causes loss of responsiveness to these agents.

DISCUSSION

Caron and Lefkowitz first used (−)-[^{3}H]DHA, a potent β-adrenergic antagonist, to identify the β-adrenergic receptor (*9*). They examined the subcellular distribution of (−)-[^{3}H]DHA binding sites in rat fat cells and found that these binding sites occurred predominantly in the plasma membrane fraction (*10*). They speculated that this distribution of (−)-[^{3}H]DHA binding sites indicated the presence of physiologically significant β-adrenergic receptors in the plasma membrane. Furthermore, they demonstrated functional interactions between the pure β-adrenergic receptor and a pure stimulatory nucleotide binding protein belonging to the adenylate cyclase system (*11*).

However, they did not examine the relationship between (−)-[^{3}H]DHA binding sites and lipolysis in fat cells. Dax and Partilla suggested that the binding of (−)-[^{3}H]DHA to adipocyte membranes involved both β-receptor and non-receptor compartments (*12*). It is now well established that three β-adrenoceptor subtypes (β_1, β_2-, and β_3-) coexist in fat cells and adipocyte precursors of various species (*e.g.*, rat, hamster, dog, rabbit, garden dormouse), as well as in the mouse 3T3-F442A preadipose cell line and probably all the other murine preadipose cell lines (*1*). Bahouth and Malbon reported that the β_1-selective antagonist CGP-20712A provided a greater degree of inhibition of catecholamine-stimulated lipolysis in rat fat cells than the β_2-selective antagonist ICI-118, 551 (*13*). Based on this result, they concluded that the character of rat fat cell β-adrenergic receptor was solely β_1. On the other hand, Lafontan and Berlan suggested that BRL 37344 acted solely and isoproterenol acted predominantly through β_3-adrenergic receptor in rat fat cells (*1*).

The purpose of the present investigation, however, was not to clarify the β-adrenoceptor subtypes involved in lipolysis in rat fat cells, but to confirm the participation of β-adrenoceptors in the lipolysis. All these β-adrenoceptors are believed to stimulate lipolysis in fat cells through production of cyclic AMP (*8*). If catecholamine-induced lipolysis in fat cells is not mediated through cyclic AMP-dependent activation of HSL, these β-adrenoceptors may not participate in the lipolysis. Although experiments on β-adrenoceptors have usually been done with plasma membrane preparations, it was necessary to use fat cells for examining the relationship between the receptors and lipolysis in the present investigation (*10*).

Cabelli and Malbon first examined specific binding sites for (−)-[^{3}H]DHA on isolated fat cells (*8*). They found that specific (−)-[^{3}H]DHA binding was proportional to cell number over the range of 0.5 to 4×10^5 cells/ml with 10 nM (−)-[^{3}H]DHA. In order to examine the relationship between β-adrenoceptors and lipolysis, a substance which was concerned in both of them was required. In this connection, (−)-alprenolol, which displaced (−)-[^{3}H]DHA binding to rat fat cells, completely inhibited norepinephrine-induced lipolysis in these cells at 10^{-5} M (Fig. 1). Therefore, the present investigation was focused on the problem of whether or not the specific (−)-[^{3}H]DHA binding sites which are displaced by 10^{-4} M (−)-alprenolol, are involved in norepinephrine-induced lipolysis in rat fat cells (Fig. 2).

After incubation of fat cells with 10 nM (−)-[^{3}H]DHA in the presence or absence of 10^{-4}M (−)-alprenolol, these cells were used for the preparation of lipid droplets. The specific (−)-[^{3}H]DHA binding component in the fat cells was transferred to the solubilized supernatant during preparation of the lipid droplets, in which no specific binding was found (Table I). (−)-[^{3}H]DHA is known to be a highly lipophilic ligand which diffuses easily across the plasma membrane and becomes entrapped within the cell in such a way that nonspecific binding increases (*14*). It seems likely that (−)-[^{3}H]DHA may be incorporated into the lipid droplets more easily than (−)-alprenolol. This may be the reason why the penetrated radioligand in the lipid droplets is identical both in the absence and presence of (−)-alprenolol. Using these lipid droplets which lack specific binding sites of (−)-[^{3}H]DHA, norepinephrine-induced lipolysis was examined in a cell-free system. Norepinephrine was found to induce lipolysis in the cell-free system consisting of specific (−)-[^{3}H]DHA-binding-depleted lipid droplets and plasma membrane-depleted HSL (Fig. 3). The maximal rate of lipolysis produced by the hormone was 4.46 μEq of free fatty acid/ml of packed lipid droplets/h, which corresponded to 72% of the maximal rate of lipolysis in fat cells (Figs. 1 and 3). The result suggests that the β-adrenergic receptor identified by (−)-[^{3}H]DHA may not participate in norepinephrine-induced lipolysis in fat cells.

The present experiments also suggest that some other β-receptor concerned with lipolysis may exist in this cell-free system. The norepinephrine-induced lipolysis in the cell-free system was not inhibited by phenoxybenzamine (an α-blocker), but was by propranolol and (−)-alprenolol (β-blockers) (Table II). In the cell-free system consisting of the lipid droplets and HSL, sonication of the droplets caused an increase in lipolysis in the absence of norepinephrine and loss of responsiveness to the lipolytic action of norepinephrine and the anti-lipolytic action of propranolol or alprenolol (Table II and Fig. 4).

Previously, we clarified that this increase in lipolysis after sonication of the lipid droplets was due to a decrease in the phosphatidylcholine concentration on the surface of the lipid droplets (*15*). Furthermore, we suggested that phospholipase C-sensitive phospholipid and trypsin-sensitive protein on the surface of the lipid droplets might participate in the new β-receptor on lipolysis of fat cells

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(*16*). The fact that sonication of the lipid droplets caused loss of responsiveness to norepinephrine and β-blockers, suggests that the lipid droplets may be closely related to the new β-receptor concerned with the lipolysis in the cell-free system.

In conclusion, specific (−)-[^{3}H]DHA binding sites in rat fat cells may not participate in the norepinephrine-induced lipolysis, and a different receptor concerned with lipolysis may exist with close relation to the lipid droplets in the fat cells. Experiments are now in progress to identify this receptor.

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REFERENCES

1. Lafontan, M. and Berlan, M. (1993) Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* **34**, 1057-1091
2. Okuda, H., Morimoto, C., and Tsujita, T. (1994) Effect of substrates on the cyclic AMP-dependent lipolytic reaction of hormone-sensitive lipase. *J. Lipid Res.* **35**, 1267-1273
3. Ninomiya, H., Morimoto, C., Tsujita, T., Sumida, M., and Okuda, H. (1990) Biomodulator-mediated susceptibility of endogenous lipid droplets from rat adipocytes to hormone-sensitive lipase. *Biochem. Med. Metab. Biol.* **43**, 112-127
4. Okuda, H., Morimoto, C., and Tsujita, T. (1992) Relationship between cyclic AMP production and lipolysis induced by forskolin in rat fat cells. *J. Lipid Res.* **33**, 225-231
5. Chen, R.F. (1967) Removal of fatty acids from serum albumin by charcoal treatment. *J. Biol. Chem.* **242**, 173-181
6. Rodbell, M. (1964) Metabolism of isolated fat cells. *J. Biol. Chem.* **239**, 375-380
7. Okuda, H., Tsujita, T., and Kinutani, M. (1986) Studies on a protein kinase inhibitor-insensitive, phospholipase C-sensitive pathway of lipolysis in rat adipocytes. *Pharmacol. Res. Commun.* **18**, 877-893
8. Cabelli, R.J. and Malbon, C.C. (1979) Characterization of (−)-[^{3}H]dihydroalprenolol binding sites on isolated rat fat cells. *J. Biol. Chem.* **254**, 8903-8908
9. Caron, M.G. and Lefkowitz, R.J. (1976) Solubilization and characterization of the β-adrenergic receptor binding sites of frog erythrocytes. *J. Biol. Chem.* **251**, 2374-2384
10. Williams, L.T., Jarett, L., and Lefkowitz, R.J. (1976) Adipocyte β-adrenergic receptors. *J. Biol. Chem.* **251**, 3096-3104
11. Cerione, R.A., Codina, J., Benovic, J.L., Lefkowitz, R.J., Birnbaumer, L., and Caron, M.G. (1984) The mammalian β_2-adrenergic receptor: Reconstitution of functional interactions between pure receptor and pure stimulatory nucleotide binding protein of the adenylate cyclase system. *Biochemistry* **23**, 4519-4525
12. Dax, E.M. and Partilla, J.S. (1982) Adrenergic ligand liposolubility in membranes: Direct assessment in a beta-adrenergic binding system. *Mol. Pharmacol.* **22**, 5-7
13. Bahouth, S.W. and Malbon, C.C. (1988) Subclassification of beta-adrenergic receptors of rat fat cells: A reevaluation. *Mol. Pharmacol.* **34**, 318-326
14. Lacasa, D., Mauriége, P., Lafontan, M., Berlan, M., and Guidicelli, Y. (1986) A reliable assay for beta-adrenoceptors in intact isolated human fat cells with a hydrophilic radioligand, [^{3}H]-CGP-12177. *J. Lipid Res.* **27**, 368-376
15. Okuda, H., Morimoto, C., and Tsujita, T. (1994) Role of endogenous lipid droplets in lipolysis in rat adipocytes. *J. Lipid Res.* **35**, 36-44
16. Morimoto, C., Tsujita, T., and Okuda, H. (1990) Propranolol-sensitive and phenoxybenzamine-insensitive binding of norepinephrine to endogenous lipid droplets from rat adipocytes. *Biochem. Med. Metab. Biol.* **44**, 126-134

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