Effect of epinephrine and other lipolytic agents on intracellular lipolysis and lipoprotein lipase activity in 3T3-L1 adipocytes

Sidney S. Chernick, Peter M. Spooner,* Mary M. Garrison, and Robert O. Scow

Endocrinology Section, Laboratory of Cellular and Developmental Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and Division of Extramural Affairs,* National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205

Abstract 3T3-L1 adipocytes were used to test the hypothesis that hormone-sensitive lipolysis and lipoprotein lipase activity might be regulated in a reciprocal manner. Intracellular lipolysis was stimulated by catecholamine, dibutyryl cAMP, and ACTH, but not by glucagon. The effects of epinephrine on lipolysis were blocked by the beta-antagonist propanolol but not by the alpha-antagonist phentolamine. Hormone-stimulated lipolysis was not changed by acute (45 min) or chronic (2 days) treatment of the cells with insulin whereas the latter treatment augmented lipoprotein lipase activity about fivefold. Epinephrine did not affect the lipoprotein lipase activity of insulin-stimulated cells. Withdrawal of glucose from the medium decreased lipoprotein lipase activity and the effect of epinephrine on lipolysis. Effects of lipolytic agents on activity of lipoprotein lipase were variable and concentration-dependent. Lipoprotein lipase activity was decreased only by concentrations of epinephrine greater than those inducing maximal intracellular lipolysis, and the decrease in activity occurred about 30 min after the increase in glycerol release. There seems to be no relationship between the level of activity of lipoprotein lipase and the maximal rate of hormone-stimulated lipolysis in 3T3-L1 cells. Unlike in adipose tissue and adipocytes of rats, hormone-stimulated lipolysis and lipoprotein lipase activity in murine 3T3-L1 adipocytes appear to be regulated independently. —**Chernick, S. S., P. M. Spooner, M. M. Garrison, and R. O. Scow.** Effect of epinephrine and other lipolytic agents on intracellular lipolysis and lipoprotein lipase activity in 3T3-L1 adipocytes. *J. Lipid Res.* 1986. **27:** 286–294.

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Two distinct lipolytic systems are involved in the movement of fatty acids in adipose tissue (1). One involves transfer of fatty acids from triacylglycerol of chylomicrons and VLDL in blood to adipocytes and is catalyzed by lipoprotein lipase acting at luminal surfaces of capillaries (2), while the other involves transfer of fatty acids, in the opposite direction, from triacylglycerol in adipocytes to plasma albumin in capillaries and is catalyzed by hormone-sensitive lipase acting in adipocytes (3). Lipoprotein lipase activity in adipose tissue is augmented by insulin and glucose (4–6), whereas hormone-sensitive lipase is stimulated by catecholamines, ACTH, and other hormones (1). The action of catecholamines on hormone-sensitive lipase is mediated through a receptor-adenyl cyclase complex which activates a protein kinase that phosphorylates the inactive enzyme (1). It has been suggested that this lipolytic cascade may also regulate the activity of lipoprotein lipase in adipose tissue (4–6).

3T3-L1 cells differentiate in culture into multilocular fat cells with many characteristics of adipocytes from murine adipose tissue (7). They synthesize and release active lipoprotein lipase in response to insulin and glucose (8–10). They also have the hormone receptor-adenyl cyclase system by which lipolytic hormones can stimulate intracellular lipolysis (11, 12). We have used these cells to test the hypothesis that lipoprotein lipase and intracellular lipolysis may be regulated in a reciprocal manner (4–6). Our results suggest that these two lipolytic systems are regulated independently in 3T3-L1 adipocytes.

EXPERIMENTAL PROCEDURES

3T3-L1 cells were grown to confluence with 20% calf serum-culture medium[1] in 100-mm culture dishes as described earlier (10). Confluent cells were stimulated to differentiate into mature fat cells by incubating them with 0.5 mM 1-methyl-3-isobutylxanthine, 2×10^{-7} M dexamethasone, and 0.8 μM insulin for 2 days and then with 0.8 μM insulin for 6–8 days in 20% serum-culture

[1]Culture medium was composed of 25 mM glucose, 50 units/ml penicillin, and 50 μg/ml streptomycin in Dulbecco's modified Eagle's medium.

medium. Mature cells were maintained in 10% serum-culture medium up to 2 weeks. Prior to study, cells were incubated for 3 to 5 days in 3–5% calf serum-medium to lower basal lipoprotein lipase activity in the cells and, thereby, enhance the effect of insulin on lipoprotein lipase activity of cells (10). Nonetheless, there was considerable variation in cellular lipoprotein lipase activity between batches of cells.

Determination of lipoprotein lipase activity

Lipoprotein lipase activity was used as a measure of lipoprotein lipase in cells and medium. Lipoprotein lipase activity in incubation media was measured immediately on aliquots of media filtered through 0.2-μM "Millex" membrane filters. Cellular lipoprotein lipase activity was determined on dried defatted powder of cells (9, 10). Briefly, cells were scraped from culture dishes with ice-cold 50 mM NH_3/NH_4Cl buffer, pH 8.1, containing 3 units/ml of heparin (buffer AH). Bovine serum albumin, 5 mg in 0.1 ml of H_2O, was added and the cell suspension was sonicated 15 sec at 4°C before taking aliquots for DNA analysis (13). The remaining sonicate was added to acetone at −86°C and centrifuged, and the precipitate was sequentially washed with acetone, acetone–diethyl ether 1:1 (v/v), and diethyl ether at room temperature. Cell powders were dried and stored under reduced pressure. For enzyme assay, the powders were resuspended in buffer AH and the soluble lipoprotein lipase activity was determined immediately using an emulsion of tri-[9,10-^{3}H]oleoylglycerol and lecithin activated with serum from fasted rats (10). Each extract was assayed in duplicate. One unit of lipoprotein lipase activity represents the release of 1 μmol of fatty acid per min at 37°C. Lipolytic activity measured in the incubation medium and cell powders was due to lipoprotein lipase since pH optimum was 8.3 and activity was inhibited more than 80% by inclusion of 1 M NaCl or omission of serum in the assay mixture.

Determination of intracellular lipolysis

Intracellular lipolysis was measured by the amount of glycerol released into the incubation medium. After incubation, media (3 ml) were decanted from the culture dishes, rapidly cooled to 0°C, and assayed immediately, or after storage at 0°C for 1–2 days. Duplicate aliquots (50 μl) were analyzed for free glycerol by the fluorimetric procedure of Chernick (14). Fatty acids were determined in 1-ml aliquots by extraction into hexane and microtitration with 0.01 N NaOH (15). Corrections for appropriate medium blanks were applied to glycerol and fatty acids assays.

Preparation of fatty acid-enriched medium

Aliquots of an oleic acid solution in hexane were evaporated to dryness in Erlenmeyer flasks with the aid of N_2. An amount of 4% albumin-culture medium was added to yield the desired oleic acid–albumin ratio and the mixture was stirred with a magnetic bar at 37°C for 1–2 hr. When oleic acid appeared to be in solution, the medium was filtered through a 0.2-μm pore filter and gassed with 95% O_2–5% CO_2. Aliquots of medium were taken for protein and fatty acid analyses to determine the oleic acid:albumin ratio.

Materials

Cell culture media were prepared by the National Institutes of Health Media Unit. Calf serum (lots E-181017 and R-990323) from Grand Island Biological Co., Grand Island, NY, was selected for its ability to promote a high and uniform degree of adipose conversion of the cells. The following compounds were obtained from the sources indicated: (−) propranolol, Ayerst Laboratories, NY; phentolamine mesylate, Ciba Pharmaceuticals, Summit, NJ; and isoproterenol bitartrate, Sterling Winthrop Research Institute, Rensselaer, NY. Epinephrine, norepinephrine bitartrate, porcine ACTH (156 U/mg), theophylline, dibutyryl 3'-5' cyclic AMP, oleic acid, alpha glycerol phosphate dehydrogenase, glycerol kinase, ATP, and NAD^+ were purchased from Sigma, St. Louis, MO. Crystalline porcine zinc glucagon and insulin were kindly provided by Eli Lilly, Indianapolis, IN. Albumin crystallized from bovine serum was purchased from Armour Pharmaceuticals, Phoenix, AZ. Prostaglandin E_1 was donated by Upjohn Co., Kalamazoo, MI.

RESULTS

Effects of lipolytic agents on intracellular lipolysis and cellular lipoprotein lipase activity

Hormonal specificity. Catecholamines and other agents that stimulate lipolysis in adipocytes (1, 16) were tested for their acute effects on release of glycerol from intracellular triacylglycerol and on cellular lipoprotein lipase activity (**Table 1**). Mature 3T3-L1 fat cells with relatively low lipoprotein lipase activity (0.5 unit/mg DNA) were incubated for 45 min with 10^{-6} M epinephrine or norepinephrine. Both hormones stimulated release of glycerol into the medium three- to fourfold, but had no effect on cellular lipoprotein lipase activity. Similar responses were evoked by isoproterenol at 10^{-7} M. Stimulation of glycerol release by 10^{-6} M epinephrine was reduced by the beta-antagonist propranolol at 10^{-5} M but not by the alpha-antagonist phentolamine at 10^{-5} M, whereas lipoprotein lipase activity was not altered significantly by either antagonist. ACTH increased intracellular lipolysis without any significant effect on lipoprotein lipase activity. High concentrations of prostaglandin E_1 (10^{-5} M), theophylline (10^{-3} M), and dibutyryl cyclic AMP (10^{-4} M) each doubled glycerol production with variable small effects on

TABLE 1. Acute effects of hormones and other agents on intracellular lipolysis and lipoprotein lipase activity in 3T3-L1 cells

Additions to Medium		Glycerol Released to Medium	Cellular Lipoprotein Lipase Activity
Substance	Conc.		
		μmol/hr per mg DNA	*unit/mg DNA*
None		5.5 ± 0.4	0.55 ± 0.04
Epinephrine	10^{-6}	25.1 ± 0.2[a]	0.51 ± 0.03
Norepinephrine	10^{-6}	20.2 ± 1.1[b]	0.51 ± 0.04
Isoproterenol	10^{-7}	26.5 ± 0.6[a]	0.46 ± 0.04
Epinephrine +	10^{-6}	8.1 ± 0.3[c]	0.38 ± 0.04
Propanolol	10^{-5}		
Epinephrine +	10^{-6}	21.6 ± 2.3[b]	0.39 ± 0.04
Phentolamine	10^{-5}		
Adrenocorticotropin	5×10^{-6}	22.6 ± 0.4[a]	0.40 ± 0.04
Prostaglandin E_1	10^{-5}	9.0 ± 0.3[b]	0.41 ± 0.03
Theophylline	10^{-3}	10.0 ± 0.8[b]	0.34 ± 0.06
Dibutyryl cAMP	10^{-4}	8.5 ± 0.1[b]	0.53 ± 0.04
Glucagon	10^{-6}	5.3 ± 0.1	0.47 ± 0.04

Mature 3T3-L1 adipocytes preincubated in 5% serum-culture medium for 5 days were incubated for 45 min at 37°C with various substances in 4% albumin-culture medium. Glycerol content of the incubation medium and lipoprotein lipase activity in cells were determined at the end of incubation. Results are means ± SE of data from two experiments, each consisting of two to four plates per point (n = 4–8).

[a]Different from control, $P < 0.001$.

[b]Different from control, $P < 0.01$.

[c]Different from control, $P < 0.05$.

lipoprotein lipase activity. Glucagon at concentrations between 10^{-4} M and 10^{-8} M had no effect on intracellular lipolysis or lipoprotein lipase activity in 3T3-L1 cells (data not shown). The glucagon preparations used in the present study were capable of stimulating adenyl cyclase activity in rat hepatocytes.

Concentration dependence. The response of mature 3T3-L1 cells with relatively high lipoprotein lipase activity (2.0 unit/mg DNA) to various concentrations of epinephrine for 60 min is shown in **Table 2.** Increased intracellular lipolysis was apparent at 10^{-8} M epinephrine and maximal at 10^{-6} to 10^{-4} M. Graphic interpolation of these data indicated that half-maximal stimulation occurred at about 2×10^{-7} M epinephrine. Lipoprotein lipase activity in the cells, however, was not affected by epinephrine at these concentrations. Epinephrine at higher concentrations, $1\text{–}5 \times 10^{-3}$ M, produced a minimally stimulated intracellular lipolysis and decreased lipoprotein lipase

TABLE 2. Acute effects of various amounts of epinephrine and dibutyryl cyclic AMP on intracellular lipolysis and lipoprotein lipase activity in 3T3-L1 cells

Additions to Medium		Glycerol Released to Medium	Cellular Lipoprotein Lipase Activity
Substances	Conc.		
	M	*μmol/hr per mg DNA*	*unit/mg DNA*
None		5.3 ± 0.3	1.96 ± 0.2
Epinephrine	10^{-8}	8.8 ± 0.4[b]	1.99 ± 0.4
Epinephrine	10^{-7}	16.8 ± 0.4[a]	1.90 ± 0.4
Epinephrine	10^{-6}	25.3 ± 0.6[a]	1.31 ± 0.2
Epinephrine	10^{-5}	24.4 ± 0.5[a]	1.51 ± 0.2
Epinephrine	10^{-4}	22.0 ± 0.4[a]	1.57 ± 0.3
Epinephrine	10^{-3}	16.1 ± 0.4[a]	0.91 ± 0.1[b]
Epinephrine	5×10^{-3}	9.0 ± 0.2[b]	0.71 ± 0.1[b]
Dibutyryl cAMP	10^{-3}	28.2 ± 1.6[a]	1.33 ± 0.1[c]
Dibutyryl cAMP	5×10^{-3}	23.1 ± 1.2[a]	1.00 ± 0.2[c]

Mature 3T3-L1 adipocytes preincubated in 5% serum-culture medium for 2 days were incubated for 60 min at 37°C with various substances in 4% albumin-culture medium. Results are means ± SE of data from two experiments, each consisting of two to four plates per point (n = 4–8).

[a]Different from control, $P < 0.001$.

[b]Different from control, $P < 0.01$.

[c]Different from control, $P < 0.05$.

activity, whereas dibutyryl cyclic AMP at the same concentrations increased glycerol released as it decreased cellular lipoprotein lipase activity (Table 2). Dibutyryl cyclic AMP at a lower concentration, 10^{-4} M, had no effect on lipoprotein lipase activity (Table 1).

Time course. The time course of the effect of 10^{-6} M epinephrine on intracellular lipolysis and cellular lipoprotein lipase activity is given in **Fig. 1.** Epinephrine doubled the rate of release of glycerol by these cells throughout the 60-min incubation, whereas it had a small lowering effect on lipoprotein lipase activity after 30 min.

Lack of effect of epinephrine on lipoprotein lipase release

Previously, we observed that insulin increased release of active lipoprotein lipase from 3T3-L1 cells into the incubation medium (10). Epinephrine, from 10^{-8} to 10^{-6} M, produced no detectable changes in release of active lipoprotein lipase to medium in 30 or 60 min, although it increased glycerol release threefold in the same basal and insulin-treated cells. Thus the increase in release of lipoprotein lipase activity induced by insulin was not altered by stimulation of lipolysis by epinephrine (data not given).

Acute and chronic effects of insulin on intracellular lipolysis and cellular lipoprotein lipase activity

Mature 3T3-L1 cells were incubated for 2 days in 5% serum–culture medium that contained less than 10^{-12} M insulin. The cells were then incubated with or without 10^{-7} M insulin in the same kind of medium for 2 more days (**Fig. 2**). The cells were washed and incubated for 45 min at 37°C in 4% albumin–culture medium with or without 10^{-6} M epinephrine, or with both epinephrine and 10^{-7} M insulin. Although incubation of cells with insulin for 2 days increased basal glycerol release, it did not alter epinephrine-stimulated intracellular lipolysis. Addition of insulin to epinephrine-stimulated cells had little effect on glycerol release. Thus, insulin treatment for 2 days increased basal but not epinephrine-stimulated lipolysis, and insulin treatment for 45 min had little effect on hormone-stimulated lipolysis.

Cells incubated with insulin for 2 days contained 4–5 times more lipoprotein lipase activity than control cells. Incubation with epinephrine, or epinephrine and insulin had no effect on lipoprotein activity in either insulin-untreated cells or cells treated with insulin for 2 days (Fig. 2).

Insulin treatment for 45 min reduced fatty acid release and increased fatty acid re-esterification in cells incubated with epinephrine. Insulin treatment for 2 days resulted in virtual suppression of fatty acid release and nearly total reesterification of fatty acids produced by intracellular lipolysis.

Effect of acute withdrawal of glucose on response of 3T3-L1 cells to epinephrine and insulin

Epinephrine at 10^{-6} M stimulated lipolysis, but had no effect during 45 min on activity of lipoprotein lipase in cells incubated with 25 mM glucose (**Fig. 3**). Insulin, from 10^{-11} to 10^{-7} M, had no effect on either intracellular lipolysis or lipoprotein lipase activity in cells incubated with epinephrine and glucose, but it markedly increased reesterification of fatty acids produced by lipolysis. The half maximal effect of insulin on reesterification occurred at 5×10^{-10} M.

Acute withdrawal of glucose for 45 min lowered basal lipoprotein lipase activity, but did not affect intracellular lipolysis in untreated cells (Fig. 3). It decreased the effect of epinephrine on lipolysis and lowered cellular lipoprotein lipase in epinephrine-treated cells. Glucose withdrawal did not change the effect of insulin on either lipolysis or lipoprotein lipase activity in epinephrine-treated cells, but it did block the effects of insulin on fatty acid release and reesterification.

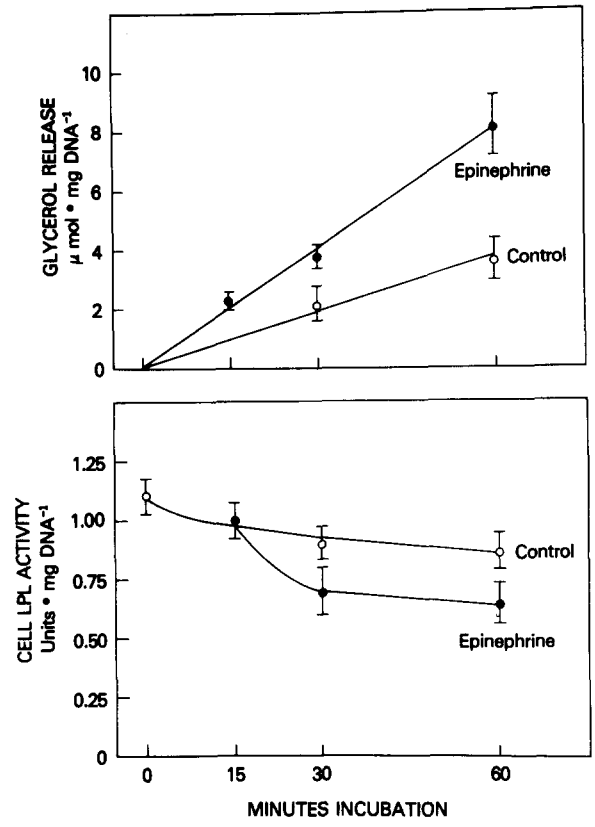

Fig. 1. Time course of effect of epinephrine on intracellular lipolysis and cellular lipoprotein lipase activity. Mature 3T3-L1 fat cells preincubated in 5% serum-culture medium for 3 days were incubated with or without 5×10^{-6} M epinephrine in 4% albumin-culture medium. Intracellular lypolysis was measured by the amount of glycerol released into the medium. Release of glycerol into the medium and cellular lipoprotein lipase (LPL) activity were determined at 0, 15, 30, and 60 min. Results are means ± SE of duplicate determinations on three or four plates of cells.

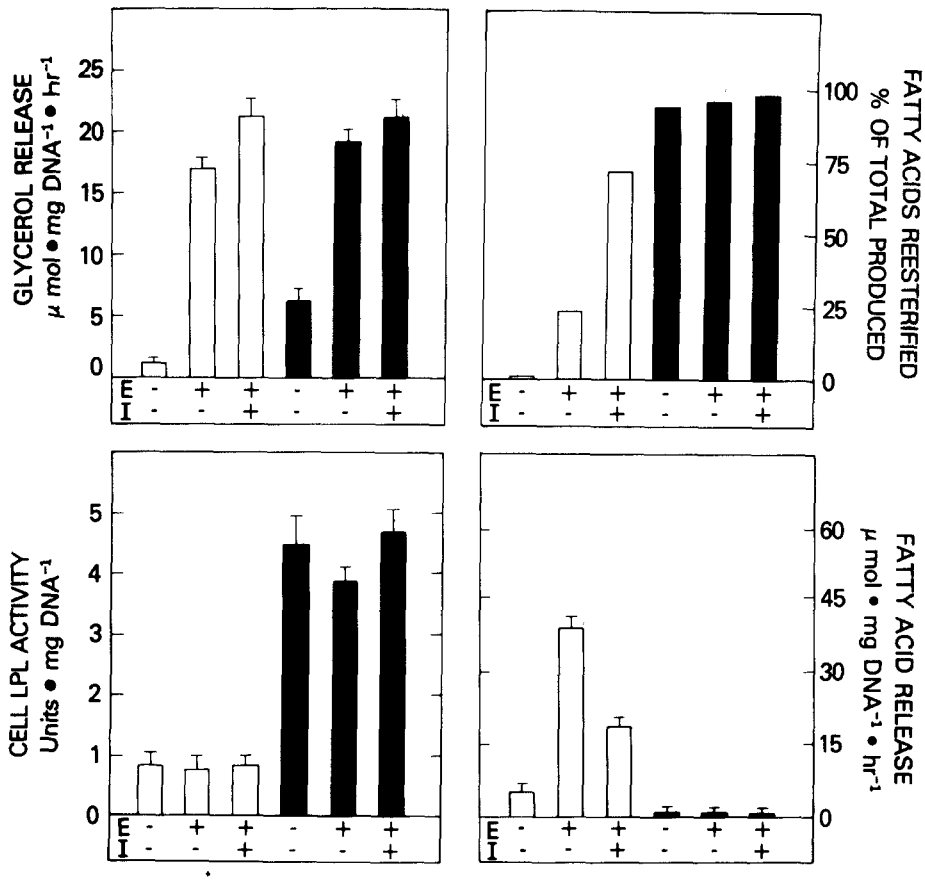

Fig. 2. Effects of insulin and epinephrine on intracellular lipolysis, reesterification of fatty acids and cellular lipoprotein lipase activity. Mature 3T3-L1 adipocytes were preincubated 2 days in 5% serum-culture medium and another 2 days with or without 10^{-7} M insulin in the same kind of medium. The cells were then washed twice with phosphate-buffered saline solution at pH 5.2 (10) and incubated 45 min with or without 10^{-6} M epinephrine (E) and with or without 10^{-7} M insulin (I) in 4% albumin-culture medium. Cells preincubated with insulin are indicated by black bars, and those preincubated without insulin are indicated by white bars. The amount of fatty acids reesterified was calculated as the difference between the amount of fatty acids produced by lipolysis (3 × mol of glycerol release = mol of fatty acids produced) and the amount of fatty acids recovered in the medium. Results are means ± SE of five determinations.

The data in Figs. 2 and 3 indicate that, in our experiments with 3T3-L1 cells, changes in intracellular concentrations of ATP, free fatty acids, and acyl CoA, associated with reesterification of fatty acids, had very little effect on the activity of lipoprotein lipase or the lipolytic response to epinephrine.

Effects of exogenous fatty acids

To determine whether fatty acids have a role in the small reduction in cellular lipoprotein lipase activity elicited by epinephrine (17), 3T3-L1 adipocytes were incubated with different amounts of oleic acid bound to albumin. **Fig. 4** shows that increasing the molar ratio of oleic acid to albumin to 6 reduced the rate of epinephrine-stimulated lipolysis by 50%, but had no effect on lipolysis in untreated control cells. Cellular lipoprotein lipase activity, in contrast, was decreased only 10–15% in both epinephrine-treated and control cells when the molar ratio of fatty acids to albumin was increased from 3 to 5. Regardless of the ratio of oleic acid to albumin in the medium, lipoprotein lipase activity was 25–30% lower in cells treated with 5×10^{-7} M epinephrine for 45 min than in untreated cells. It is unlikely, therefore, that intracellular concentration of fatty acids, per se, is a major factor in the decreased activity of lipoprotein lipase in cells treated with epinephrine.

DISCUSSION

Intracellular lipolysis in 3T3-L1 cells was stimulated in our studies by epinephrine, norepinephrine, and ACTH, confirming observations of others in 3T3-L1 cells and murine adipocytes (11, 12, 16). The dose-response study of epinephrine showed that this hormone had a maximal stimulatory effect on intracellular lipolysis at a concentration of 10^{-6} to 10^{-4} M (half maximal stimulation at

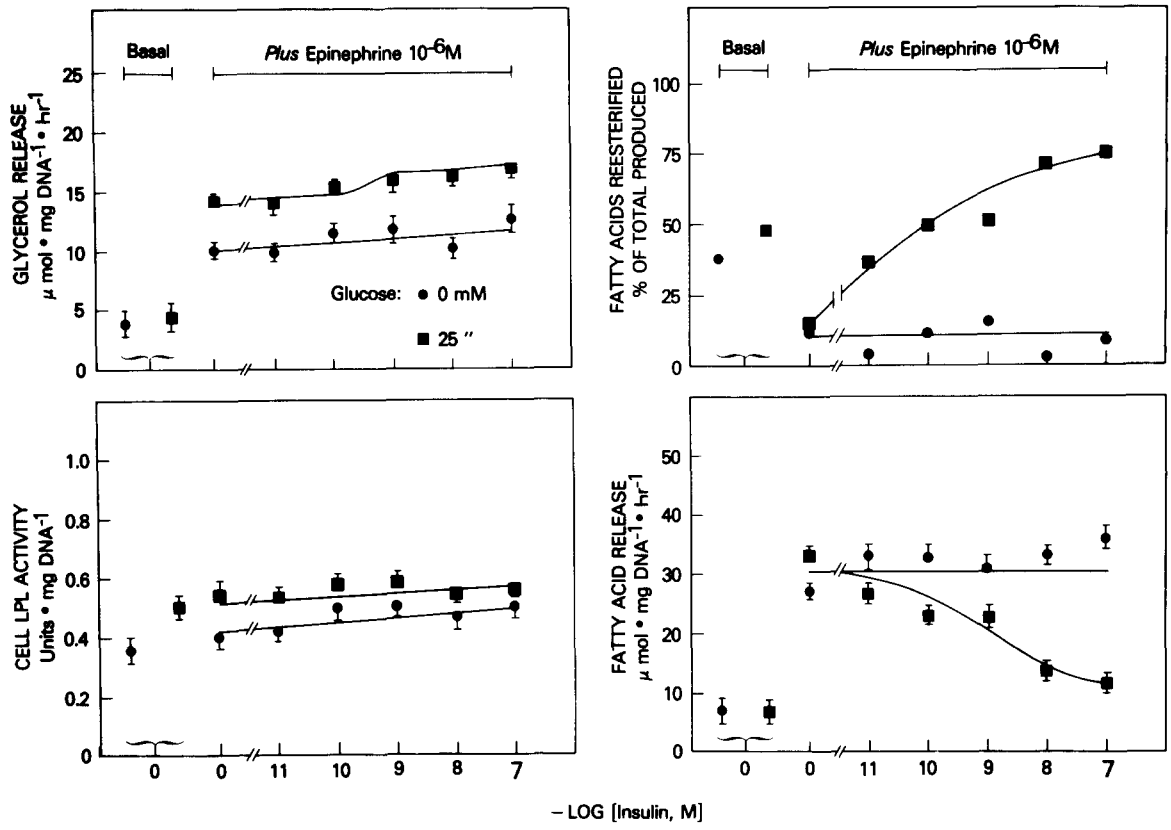

Fig. 3. Effect of acute glucose withdrawal on response of 3T3-L1 cells to epinephrine and various concentrations of insulin. Mature 3T3-L1 adipocytes were incubated for 4 days in 5% serum-culture medium, which contained 25 mM glucose. Cells were then rinsed twice with 10 ml of phosphate-buffered saline solution, pH 7.4, and incubated 45 min with 0 or 25 mM glucose, 0 or 10^{-6} M epinephrine, and various concentrations of insulin in 4% albumin-DME medium. Release of glycerol and fatty acids into the medium and cellular lipoprotein lipase activity were determined at the end of the incubation. Reesterification of fatty acids was calculated as in Fig. 2. Results are means ± SE of three determinations.

2×10^{-7} M) and had decreased effects at $1\text{-}5 \times 10^{-3}$ M. The stimulatory effect of epinephrine was reduced 30% by acute withdrawal of glucose from the incubation medium (Fig. 3). The effect of epinephrine on lipolysis in 3T3-L1 cells was blocked by the beta-antagonist propanolol, but not by the alpha-antagonist phentolamine. Isoproterenol mimicked the effect of epinephrine on lipolysis in 3T3-L1 cells, as reported by others (12). Stimulatory effects on intracellular lipolysis were also produced by dibutyryl cAMP at 10^{-4} M. Glucagon, which has lipolytic effects on human and rat adipocytes (1), did not increase intracellular lipolysis in 3T3-L1 cells. A similar lack of effect of glucagon was obtained in adipocytes isolated from mouse adipose tissue (16).

There are several examples of a physiological antagonism between insulin and epinephrine, in the regulation of glucose and fatty acid concentrations in blood, and in the regulation of glycogenesis and glycogenolysis in liver and muscle. Thus, insulin could be expected to negate the stimulation of lipolysis by catecholamine in adipose tissue. The interactions of insulin and catecholamines in the regulation of lipolysis, however, appear to be complicated (17–21). Lipolysis in rat adipocytes is both enhanced and suppressed by insulin (18–20). Desai, Li, and Angel (20) demonstrated that the bimodal effect of insulin on hormone-stimulated lipolysis was correlated positively with changes in peak intracellular cyclic AMP levels. Acute and chronic effects of insulin on isoproterenol-stimulated lipolysis were studied in 3T3-L1 cells by Olansky and Pohl (21). Insulin decreased within 2 hr the apparent affinity (K_m) for isoproterenol without affecting the maximal rate of intracellular lipolysis (V_{max}). This is in agreement with our finding that insulin had no acute effect on the rate of glycerol release evoked by 10^{-6} M epinephrine (Fig. 2). They found that treatment with insulin for 1–2 days reduced the magnitude of isoproterenol-stimulated lipolysis (V_{max}) without affecting the affinity of the cells for isoproterenol (K_m). The effect of insulin on lipolysis was maximal at 12–24 hr and markedly reduced at 48 hr. Since similar biphasic responses to insulin occurred when lipolysis was stimulated with dibutyryl cyclic AMP, the changes induced by insulin were distal to the production of cyclic AMP. We also found no difference in hormone-stimulated lipolysis be-

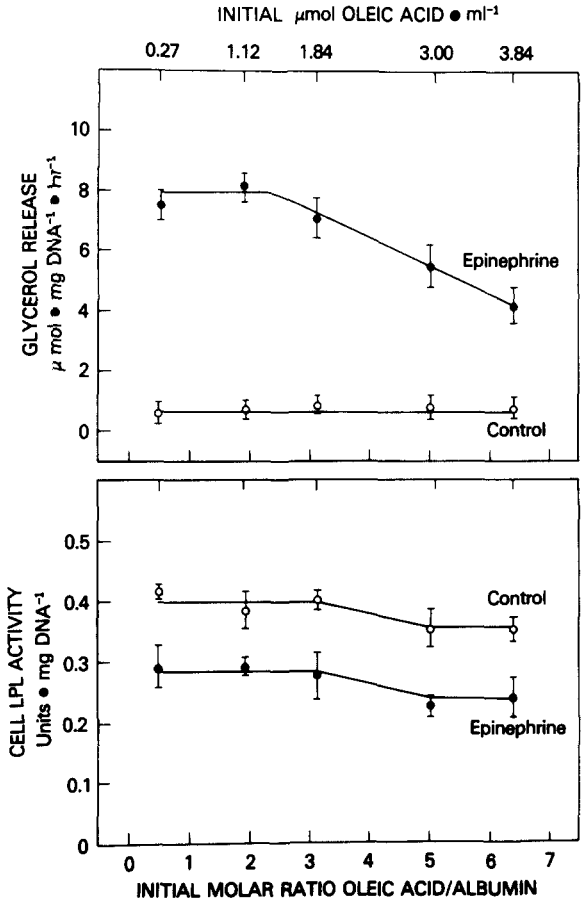

Fig. 4. Effect of medium fatty acid concentration on intracellular lipolysis and lipoprotein lipase activity in untreated and epinephrine-treated adipocytes. Mature 3T3-L1 cells preincubated in 5% serum-culture medium for 5 days were incubated 45 min with or without 5×10^{-7} M epinephrine in 4% albumin-culture medium containing different amounts of oleic acid. Results are means ± SE of duplicate determinations on three or four plates of cells.

tween cells treated with insulin for 2 days and those not treated with insulin (Fig. 2). We did not observe, however, an anti-lipolytic effect of insulin as reported by Olensky and Pohl (21). The differences between their findings and ours could be due to the way cells were treated prior to experiment, the concentrations of hormones used, or the duration of hormone treatment. Nonetheless, there are several points of concurrence between our findings as noted above.

Responsiveness of the hormone-sensitive lipase system to catecholamines and lipoprotein lipase activity both increase during differentiation of 3T3-L1 fibroblasts to adipocytes (9, 11). Similar changes also occur during maturation of rat preadipocytes to adipocytes in primary culture (22). The levels of activity of hormone-sensitive lipase and lipoprotein lipase in isolated adipose tissue and adipocytes from adult rats, however, appear to vary in a reciprocal manner as a function of nutritional and hormonal status (1, 3, 5, 17). Ashby and Robinson (4) and Robinson et al. (17) reported that lipoprotein lipase activity in incubated adipose tissue of starved rats increased in the presence of insulin and glucose, and this was potentiated by glucocorticoids. Lipolytic agents, such as catecholamines and dibutyryl cAMP, prevented the response to insulin and the potentiating effect of glucocorticoids. Lipolytic agents also inhibited or reversed the rise in lipoprotein lipase activity in tissues of starved rats incubated with cycloheximide and glucose. Lipoprotein lipase activity in tissues treated with lipolytic agents was 30–50% less than that in untreated tissues. On the basis of his research, Robinson et al. (17) concluded that catecholamines mediate inactivation of adipose tissue lipoprotein lipase prior to secretion from the adipocytes. The inactivation involves inhibition of both protein synthesis and post-translational glucose-dependent mechanisms, but does not appear to operate directly through a cAMP-dependent kinase. Either elevated concentrations of free fatty acids and/or lysosomal mechanisms could be implicated in the reduction of lipoprotein lipase activity by catecholamines (17).

In experiments with fat cells from fed rats, Patten (6) observed that lipoprotein lipase activity decreased 10% in 1 hr when cells were incubated with glucose and insulin and 50% when cells were without glucose and insulin. These findings are similar to our observation that 3T3-L1 cells require insulin and glucose in the medium to maintain lipoprotein lipase activity (10). Dibutyryl cAMP in the incubation medium decreased lipoprotein lipase activity 50% in fat cells incubated with glucose and insulin, but had no effect in the absence of glucose and insulin. Dibutyryl cAMP stimulated intracellular lipolysis in both cases. The effects of lipolytic agents on lipoprotein lipase activity in rat adipose tissues and cells were manifested only when activity of the lipase was being increased or maintained with insulin and glucose (4, 6). The minimal amount of dibutyryl cAMP needed to stimulate glycerol release was less than that needed to lower lipoprotein lipase activity in the cells, and stimulation of hormone-sensitive lipase occurred well before the reduction in lipoprotein lipase activity. Lipolysis in the presence of glucose and insulin reduced cellular ATP content more readily than in their absence, despite the well-known augmentation of glucose metabolism by insulin in fat cells. Patten (6) concluded that stimulation of lipolysis decreased cellular ATP due to its consumption during reesterification of fatty acids and, therefore, protein synthesis in the fat cells was limited. The decrease in protein synthesis, coupled with the rapid decay in activity of lipoprotein lipase, led to the observed reduction of this enzyme during lipolysis (6).

In 3T3-L1 cells the activity of lipoprotein lipase (0.5 and 2 units per mg DNA, Tables 1 and 2) was not related to the maximal activity of epinephrine-stimulated lipolysis. However, under some conditions, catechol-

amines did lower the activity of lipoprotein lipase in the cells. This effect was variable and dependent on the amount of lipolytic agent used (Tables 1 and 2). Epinephrine at 10^{-8}–10^{-7} M stimulated glycerol production without affecting lipoprotein lipase activity; at 10^{-6}–10^{-4} M maximal stimulation of glycerol release occurred with little, if any, decrease in lipoprotein lipase activity; and at 5×10^{-3} M stimulation of lipolysis was decreased and activity of lipoprotein lipase was reduced (Tables 1 and 2). Decrease in lipoprotein lipase activity required a longer incubation with epinephrine than was required to stimulate glycerol release (Fig. 1). To test whether intracellular free fatty acids were responsible for the decrease in lipoprotein lipase activity, the molar ratio of oleic acid to albumin in the medium was adjusted from less than 1 to more than 6. At molar ratios greater than 3, glycerol release from epinephrine-stimulated cells was decreased, but even at 3.8 mM exogenous oleic acid (molar ratio 6.5) there was little effect on the lipoprotein lipase activity of 3T3-L1 cells (Fig. 4). These results agree with those shown in Fig. 3, in which intracellular fatty acids are reduced by reesterification in the presence of insulin and glucose without change in lipoprotein lipase activity. However, they do not agree with the finding of Patten (6) that stimulation of reesterification of fatty acids results in a reduction of protein synthesis and lowered lipoprotein lipase activity. Furthermore, when 3T3-L1 cells were incubated with insulin for 2 days, which increased lipoprotein lipase activity fivefold, epinephrine (10^{-6} M for 45 min) did not decrease cellular lipoprotein lipase activity. The results in Figs. 2 and 3 clearly show that neither intracellular lipopolysis nor lipoprotein lipase activity are appreciably altered by conditions favoring increased reesterification of endogenous fatty acids by 3T3-L1 cells under the conditions we used. Thus, these findings in 3T3-L1 cells do not support the hypothesis of Robinson et al. (17) or the conclusions of Patten (6) that lipoprotein lipase activity is regulated by catecholamines and/or intracellular lipolysis.

Earlier (10) we proposed that insulin regulates the activity of lipoprotein lipase in 3T3-L1 cells by three actions: *1*) insulin stimulates protein synthesis, including lipoprotein lipase; *2*) insulin increased hexose uptake and metabolism resulting in post-translational activation of the lipase; and *3*) insulin promotes the release of active lipase from the cells.

We have been unable to demonstrate that any of these actions of insulin were impaired by stimulation of intracellular lipolysis. The data presented in Table 2 and Figs. 1 and 4 suggested that, under some conditions, fatty acids, or their derivatives, might reduce the activity of cellular lipoprotein lipase by mechanisms that are not known at this time. However, we conclude that lipoprotein lipase activity and hormone-sensitive lipolysis in 3T3-L1 cells, in culture, are regulated independently.

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