Lipoprotein lipase activity is stimulated by insulin and dexamethasone in cardiomyocytes from diabetic rats

H. Stephen Ewart, Rogayah Carroll, and David L. Severson

Abstract: Type 1 diabetes mellitus reduces lipoprotein lipase (LPL) activity in the heart. The diabetic phenotype of decreased LPL activity in freshly isolated cardiomyocytes persisted after overnight culture (16 h). Total cellular LPL activity was 311 ± 56 nmol oleate released·h^{-1}·mg^{-1} cell protein in diabetic cultured cardiomyocytes compared with 661 ± 81 nmol oleate released·h^{-1}·mg^{-1} cell protein for control cultured cells. Diabetes also resulted in lower heparin-releasable (HR) LPL activity compared with control cells (111 ± 25 vs. 432 ± 63 nmol·h^{-1}·mg^{-1} cell protein). In kinetic experiments, the reduction in total cellular LPL and HR-LPL activities in cultured cells from diabetic hearts was due to a decrease in maximal velocity, with no change in apparent K_m for substrate (triolein). LPL activity in primary cultures of cardiomyocytes from control rats is stimulated by the combination of insulin (Ins) and dexamethasone (Dex). Overnight treatment of cultured cardiomyocytes from diabetic rats with Ins+Dex elicited an 84% increase in cellular LPL activity (to 572 ± 65 nmol·h^{-1}·mg^{-1} cell protein) and a 194% increase in HR-LPL activity (to 326 ± 46 nmol·h^{-1}·mg^{-1} cell protein). This stimulation occurred at subnanomolar concentrations of the hormones, but neither hormone was effective alone. The amount of immunoreactive LPL protein mass in cultured cardiomyocytes from diabetic hearts was unchanged by Ins+Dex treatment. Addition of oleic acid (60 μM) to the overnight culture medium inhibited the already reduced HR-LPL activity in diabetic cultured cells by 73% (to 30 ± 4 nmol·h^{-1}·mg^{-1} cell protein). The presence of oleic acid also reduced hormone-stimulated HR-LPL activity. Increasing the glucose concentration in the culture medium to 26 mM had no effect on total cellular LPL or HR-LPL activities.

Key words: lipoprotein lipase, cardiomyocytes, diabetes.

Résumé : Le diabète sucré de type 1 réduit l'activité de lipoprotéine lipase (LPL) dans le coeur. Le phénotype diabétique de l'activité LPL dans des cardiomyocytes fraîchement isolés s'est maintenu pendant toute la durée de la culture (16 h). L'activité LPL cellulaire totale a été 311 ± 56 nmol d'oléate libéré·h^{-1}·mg^{-1} de protéine cellulaire dans les cardiomyocytes diabétiques cultivés comparativement à 661 ± 81 nmol d'oléate libéré·h^{-1}·mg^{-1} de protéine cellulaire dans les cellules témoins cultivées. L'activité LPL libérable par héparine (LH) des cardiomyocytes diabétiques a été réduite comparativement à celle des cellules témoins (111 ± 25 contre 432 ± 63 nmol·h^{-1}·mg^{-1} de protéine cellulaire). Dans les expériences cinétiques, la diminution des activités LPL-LH et LPL cellulaire totale dans les cellules diabétiques cultivées a été attribuée à une diminution de la vitesse maximale, sans variation de K_m apparent pour le substrat (trioléine). L'activité LPL dans les cultures primaires de cardiomyocytes des rats témoins est stimulée par l'emploi d'insuline (Ins) combinée au dexaméthasone (Dex). Le traitement des cardiomyocytes cultivés des rats diabétiques pendant 16 h avec la combinaison Ins+Dex a induit une augmentation de 84% de l'activité LPL cellulaire (jusqu'à 572 ± 65 nmol·h^{-1}·mg^{-1} de protéine cellulaire) et une augmentation de 194% de l'activité LPL-LH (jusqu'à 326 ± 46 nmol·h^{-1}·mg^{-1} protéine cellulaire). Ces augmentations se sont produites à des concentrations sous-nanomolaires des hormones; toutefois, les hormones ont été inefficaces lorsqu'utilisées séparément. La quantité de protéines LPL immunoréactives dans les cardiomyocytes des coeurs diabétiques n'a pas été modifiée par le traitement avec Ins+Dex. L'addition d'acide oléique (60 μM) au milieu de culture a inhibé de 73% (jusqu'à 30 ± 4 nmol·h^{-1}·mg^{-1} protéine cellulaire) l'activité LPL-LH déjà réduite des cellules cultivées diabétiques. La présence d'acide oléique a aussi réduit l'activité LPL-LH stimulée par les hormones. L'augmentation de la concentration de glucose dans le milieu de culture à 26 mM n'a eu aucun effet sur les activités LPL-LH ou LPL témoin.

Mots clés : lipoprotéine lipase, cardiomyocytes, diabète.

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Introduction

Lipoprotein lipase (LPL; EC 3.1.1.34) catalyses the hydrolysis of triacylglycerols in lipoproteins to 2-monoacylglycerol and free fatty acids. Cardiomyocytes are the principal, if not exclusive, source of LPL activity in the adult heart (Camps et al. 1990; O'Brien et al. 1994). Active LPL is secreted by cardiomyocytes and then is translocated to the coronary

vasculature, where bound to heparan sulfate proteoglycans (HSPG) on the endothelial surface, the enzyme acts on triacylglycerol-rich lipoproteins, chylomicrons and very low density lipoproteins (VLDL) (Braun and Severson 1992*a*). LPL bound to HSPG on the cell surface of endothelial cells and cardiomyocytes can be displaced into the medium by heparin (Braun and Severson 1991, 1992*a*).

Fatty acids are the preferred metabolic substrate for the heart, and so fatty acids released by LPL are an important oxidative fuel for cardiomyocytes. LPL is subject to physiological regulation by a variety of metabolic conditions. For example, myocardial LPL activity is increased by fasting and decreased in the fed state (Braun and Severson 1992*a*). In type 1 diabetes mellitus, myocardial LPL activity is decreased. Insulin-deficient diabetes results in decreased degradation of VLDL (O'Looney et al. 1983, 1985) and reduced functional heparin-releasable LPL (HR-LPL) activity bound to the endothelium of perfused rat hearts (O'Looney et al. 1983; Braun and Severson 1992*b*). This inhibitory effect of diabetes is not due to a reduction in HSPG binding sites for LPL on the coronary endothelium of diabetic hearts (Liu and Severson 1996). In freshly isolated cardiomyocytes from acutely diabetic rat hearts, both HR-LPL and total cellular LPL (C-LPL) activities are reduced with only a small decrease in LPL protein mass; as a result, LPL specific enzyme activity is reduced, suggesting that a post-translational event results in the accumulation of inactive LPL protein (Carroll et al. 1995).

The precise set of physiological factors leading to the reduction in myocardial LPL during diabetes is unclear. Changes in metabolic substrate availability and (or) changes in hormone levels may be involved. For example, incubation of control cultured cardiomyocytes with oleic acid resulted in a decrease in HR-LPL activity similar to that seen in diabetes (Anderson et al. 1997). Although insulin administration in vivo to diabetic rats rapidly reversed the decrease in myocardial LPL activity, insulin did not stimulate LPL when added in vitro to cardiomyocytes from diabetic or control rats (Braun and Severson 1991, 1992*b*; Rodrigues et al. 1992). These observations suggest that an additional in vivo factor, possibly another hormone, may have to act with insulin to stimulate myocardial LPL activity.

We recently reported that the combination of insulin and dexamethasone has a stimulatory effect on HR-LPL and C-LPL activities following an overnight incubation with cultured cardiomyocytes from control rats (Ewart et al. 1997). In the present study we have examined whether diabetic cardiomyocytes retain their diabetic phenotype of low LPL activity following overnight primary culture and, if so, whether insulin and (or) dexamethasone still can stimulate LPL activity in these cells.

Materials and methods

Experimental animals

Male Sprague–Dawley rats (180–200 g) were obtained from local breeding sources at The University of Calgary, housed under a 12 h light : 12 h dark cycle, and allowed access to tap water and standard laboratory chow ad libitum. All procedures involving animals conformed to the principles outlined in the *Guide to the Care and Use of Experimental Animals*, from the Canadian Council on Animal Care, and were approved by the Committee on Animal Bioethics and Care at The University of Calgary.

Rats were made diabetic under methoxyflurane (Metafane; Janssen Pharmaceutica, North York, Ont.) anesthesia by a single injection of streptozotocin (100 mg/kg rat; Sigma, St. Louis, Mo.) into the tail vein. Nondiabetic controls received an equivalent volume of sodium citrate (10 mM, pH 4.5). Animals were used 5–7 days following injection. This acute and severe model of insulin-deficient diabetes results in marked hyperglycemia and hyperlipidemia (Braun and Severson 1991, 1992*b*; Rodrigues et al. 1992).

Preparation and incubation of cardiomyocytes

Ventricular cardiomyocytes were isolated under aseptic conditions by collagenase treatment as described previously (Ewart et al. 1997). Freshly isolated cells were suspended in culture medium (Joklik minimal essential medium supplemented with 25 mM $NaHCO_3$, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1 mM DL-carnitine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 0.2% w/v bovine serum albumin (BSA), pH 7.4), which had been filtered through a 0.22-μm filter. Cell viability was determined by trypan blue (0.4% in 0.9% NaCl) exclusion. Viable cells had a rod-shaped morphology with clear cross striations. Cell number was determined in duplicate using a hemocytometer. A preparation from a single heart yielded 7–9 million viable cells, which were diluted to a density of 150 000 cells/mL.

Cardiomyocytes were cultured as described previously (Ewart et al. 1997). Cells were seeded in laminin-coated six-well (35 mm) plates for measurements of LPL activity; 2 mL of culture medium was added to each well, followed by 1 mL of the myocyte suspension. For measurements of LPL mass, 5 mL of myocyte suspension was added to 10 mL of culture medium in laminin-coated 10-cm culture dishes. Following a 3-h plate-down, fresh culture medium was provided, which contained various additions: insulin (Ins, 100 nM), dexamethasone (Dex, 100 nM), or Ins plus Dex. Control incubations received an equal volume of vehicle (1 μL/mL 10 mM HCl). In some incubations, the culture medium was supplemented with glucose (final concentration 26 mM) or with oleic acid (60 μM; 2:1 molar ratio to albumin). Cells were incubated overnight (16 h) at 37°C under a humidified atmosphere of 95% O_2 – 5% CO_2. The cells were then incubated in fresh culture medium (1 mL/well or 2 mL/10-cm dish) with or without 5 U/mL heparin for 30 min. The medium from heparin-treated cells was removed for subsequent determinations of heparin releasable LPL (HR-LPL) activity. HR-LPL activity represents the fraction of total cellular LPL that is bound to the cell surface of cardiomyocytes (Braun and Severson 1991). Total cellular LPL activity (C-LPL) was determined in lysates from cardiomyocytes incubated in the absence of heparin. Cells were scraped in 0.25 mL of lysis buffer (50 mM ammonia buffer, pH 8.0, containing 0.05% Triton X-100), diluted with 1 mL of buffer A (250 mM sucrose, 10 mM Hepes, 1 mM EDTA, 1 mM dithiothreitol, pH 7.4) and then sonicated (Carroll et al. 1995). Cell lysates were stored at –80°C before assay.

Assay of LPL activity

LPL activities in cultured cardiomyocytes were determined routinely by measuring hydrolysis of a sonicated [^{3}H]triolein substrate emulsion (Rodrigues et al. 1992) except that the specific activity of the [^{3}H]triolein substrate was increased to 6 mCi/mmol (1 Ci = 37 GBq) by reducing the substrate concentration in the assay from 0.6 to 0.1 mM (Ewart et al. 1997). The standard assay contained 0.1 mM glycerol-[9,10-^{3}H]trioleate (approximately 500 000 dpm; 1 dpm = 0.0167 Bq), 25 mM Pipes (pH 7.5), 0.05% w/v essentially fatty acid free BSA, 50 mM $MgCl_2$, and 2% heat-inactivated chicken serum as the LPL activator. For assay of cell lysates, 2 U/mL heparin was also present. Medium (100 μL) or

cell lysate (50 µL) was incubated in a final volume of 400 µL for 30 min at 30°C. [^{3}H]Oleate generated in the assay was measured by liquid–liquid extraction (Rodrigues et al. 1992) and radioactive scintillation counting. All assays were performed in duplicate; LPL activity is expressed as nmol oleate released·h^{-1}·mg^{-1} protein in the sonicated cell extracts. Protein concentration was measured using a Coomassie Blue spectrophotometric assay (Spector 1978) with bovine serum albumin as standard.

To compare kinetic parameters (K_m, V_{max}) for LPL activities in both freshly isolated and cultured cardiomyocytes from control and diabetic rat hearts, substrate ([^{3}H]triolein or [^{14}C]triolein) concentrations in the assay were varied from 0.025 to 0.6 mM; [^{14}C]triolein was utilized as the substrate in some of these kinetic experiments because of lower radioactive blanks in the assay, thus increasing assay sensitivity. Cell lysates and post-heparin medium from freshly isolated cells were obtained as described previously (Carroll et al. 1995). C-LPL and HR-LPL activities in freshly isolated cells are expressed as nmol oleate released·h^{-1}·10^{-6} cells rather than nmol·h^{-1}·mg^{-1} protein because fresh cells are finally suspended in a medium containing 1% (w/v) BSA. Kinetic parameters (apparent K_m, V_{max}) were calculated using a computer program (Enzfitter) from Biosoft.

Preparation of anti-LPL antibodies

Antibodies against purified LPL were raised in egg-laying hens as described previously (Ewart et al. 1997) and affinity purified using a LPL Affigel-10 column (Ewart et al. 1997; Goers et al. 1987). Control IgY was isolated from egg yolks of pre-immune hens. A portion of the affinity-purified anti-LPL antibody was biotinylated using *N*-hydroxysuccinimidyl 6-(biotinamido)hexanoate (Vector Laboratories Inc., Burlingame, Calif.) as described previously (Ewart et al. 1997) and stored at –80°C.

ELISA for LPL

Following 30-min incubations with and without heparin, media from 10-cm dishes were removed, lyophilised, and resuspended in 0.2 mL H_2O. Cells were scraped in 1 mL of buffer A, pelleted, and sonicated in 0.2 mL of 25 mM NH_4Cl, 5 mM EDTA, 0.8% (w/v) Triton X-100, 0.04% (w/v) sodium dodecyl sulfate, 33 µg/mL heparin, and 10 µg/mL leupeptin (pH 8.2). Samples were diluted for assay in phosphate-buffered saline (PBS) containing 0.05% Tween 20, 1 mg/mL heparin, 0.4% BSA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/mL leupeptin, and 1 µg/mL pepstatin A (pH 7.5).

An ELISA for LPL mass was performed as described previously (Ewart et al. 1997). Briefly, samples were added to microtiter plates (Immulon 1) that had been coated with anti-LPL antibody. Purified bovine milk LPL (0.1 to 1.0 ng/well) served as standard for the ELISA. The plates were incubated overnight at 4°C and were then treated with biotinylated anti-LPL antibody in a second overnight incubation. Peroxidase-labelled streptavidin (Boehringer Mannheim, Dorval, Que.) treatment followed by *ortho*-phenylenediamine–H_2O_2 was used as a detection system. Absorbance at 495 nm was determined using a Bio-Rad microplate reader. LPL mass is expressed as ng/mg cell protein. This ELISA method detects both active (dimeric) and inactive (monomeric) forms of LPL (Ewart et al. 1997). LPL specific activity is expressed as mU/ng LPL protein, where 1 mU is defined as the amount of enzyme catalyzing the release of 1 nmol oleate per minute (Carroll et al. 1995).

Materials

Joklik minimal essential medium and penicillin–streptomycin were purchased from Gibco Canada (Burlington, Ont.). Collagenase was purchased from Technicon Canada Ltd. (Richmond, B.C.). Heparin was from Calbiochem (San Diego, Calif.), and Hepalean (1000 U/mL) was obtained from Organon Teknika (Toronto, Ont.). Laminin was from Becton Dickinson Labware (Bedford, Mass.). Insulin, dexamethasone, and essentially fatty acid free albumin were products of Sigma Chemical Co. Glycerol-[9,10-^{3}H]trioleate and glycerol-tri[1-^{14}C]oleate were from Amersham Canada (Oakville, Ont.).

Statistics

Results are expressed as means ± SEM, where *n* is the number of individual cultured cardiomyocyte preparations. The effect of diabetes and the various hormone treatments on HR-LPL and C-LPL activities, presented in Fig. 1, was analysed using two-way analysis of variance (ANOVA). Comparisons were made by the Dunnett's multiple comparisons test. One-way ANOVA followed by the Dunnett's multiple comparisons test or the paired *t*-test was used to determine differences among group means of the other data. Statistical significance corresponded to $p < 0.05$.

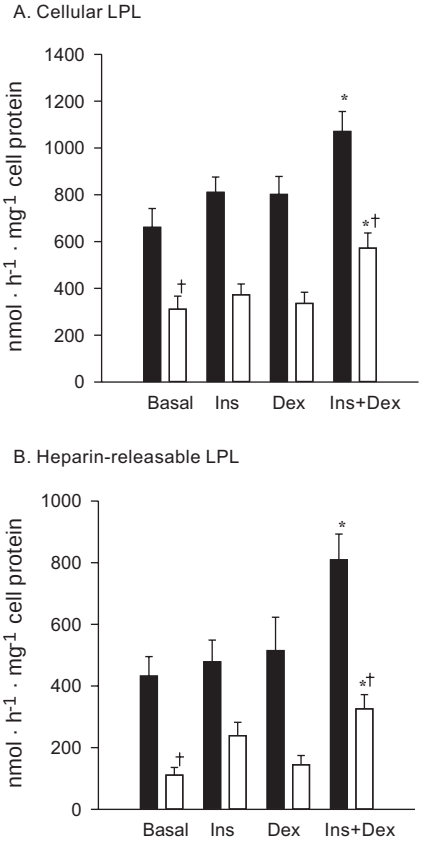
Results

Previously, we reported that LPL activity in freshly isolated cardiomyocytes from diabetic rats was reduced to approximately 50% of LPL activity in control cells (Braun and Severson 1991, 1992*b*; Carroll et al. 1995; Rodrigues et al. 1992). This phenotype of reduced LPL activity was retained by the diabetic cardiomyocytes following overnight culture (Fig. 1; basal conditions), relative to LPL activity in cultured control cells. This was evident in terms of both total cellular activity (C-LPL) and heparin-releasable activity (HR-LPL), which were reduced compared with levels in control cells by 53% (from 661 to 311 nmol·h^{-1}·mg^{-1} cell protein) and 74% (from 432 to 111 nmol·h^{-1}·mg^{-1} cell protein), respectively. The comparable decline in both total C-LPL and HR-LPL activities suggests that reduced LPL activity on the cell surface (HR-LPL) is secondary to the decline in intracellular LPL activity, but alterations in the intracellular secretory (translocation) process should be examined as a contributing mechanism to the diabetes-induced reduction in HR-LPL activity.

The kinetic mechanism responsible for the diabetes-induced reduction in LPL activities from both freshly isolated and cultured cardiomyocytes was investigated next (Fig. 2). In freshly isolated cells from diabetic hearts, the V_{max} for total cellular LPL activity was reduced by 54% (Fig. 2A) with little or no change in the apparent K_m values (107 and 127 µM, for control and diabetic cells, respectively). An even more pronounced 76% reduction in the V_{max} for HR-LPL activity in freshly isolated diabetic cardiomyocytes was observed (Fig. 2C). A similar kinetic mechanism was observed for diabetic cardiomyocytes after overnight culture. The V_{max} for C-LPL activity was reduced by 60% in diabetic cultured cardiomyocytes (Fig. 2B; from 730 to 290 nmol oleate released·h^{-1}·mg^{-1} cell protein), with no change in the apparent K_m (123 and 121 µM) for control and diabetic C-LPL activities. As noted for freshly isolated cells (Fig. 2C), the V_{max} for HR-LPL activity in diabetic cultured cells was reduced extensively by 72% (Fig. 2D; from 327 to 93 nmol·h^{-1}·mg^{-1} cell protein). Thus, the same kinetic mechanism (decreased V_{max}) is responsible for the persistent reduction in LPL activity in diabetic cardiomyocytes after overnight culture.

The apparent K_m values for sonicated triolein substrate emulsions were very similar for total C-LPL activity in cell

Fig. 1. The effect of diabetes on C-LPL and HR-LPL activities in cardiomyocytes after overnight culture without or with insulin and (or) dexamethasone. Cardiomyocytes from control (closed bars) and diabetic (open bars) rats were cultured overnight with no additions (Basal), 100 nM insulin (Ins),100 nM dexamethasone (Dex), or insulin plus dexamethasone (Ins+Dex). Results are the means ± SEM of 6 and 7 individual experiments for (A) C-LPL and (B) HR-LPL activities, respectively. Data were analysed using two-way ANOVA. The main effects of phenotype and hormone treatment significantly altered LPL activities, but acted independently (i.e., no interaction). *Significantly different from the corresponding basal activity (Dunnett's multiple comparisons test, $p < 0.05$); †significantly different from activity in control cultured cells (Student's *t*-test, $p < 0.05$).



lysates and for HR-LPL activity in the post-heparin medium from both freshly isolated and cultured cells (Fig. 2). In contrast, Ben-Zeev et al. (1981) reported that the apparent K_m for LPL in post-heparin perfusates from rat hearts was only one-tenth of the K_m for residual LPL in heart homogenates. Differences in preparation of substrate emulsions and in extraction conditions for particulate LPL in heart homogenates and cell lysates probably account for this discrepancy.

Addition of 100 nM insulin (Ins) or 100 nM dexamethasone (Dex) individually to the overnight culture medium had no effect on C-LPL or HR-LPL activities in diabetic cardiomyocytes, but the combination of insulin plus dexamethasone (Ins+Dex) significantly increased C-LPL by 84% (to 572 nmol·h^{-1}·mg^{-1} cell protein) and HR-LPL by 194% (to 326 nmol·h^{-1}·mg^{-1} cell protein) over the corresponding basal values (Fig. 1). In control cultured cardiomyocytes Ins+Dex treatment increased C-LPL and HR-LPL activities by 62 and 87%, respectively (Fig. 1), in agreement with previous findings (Ewart et al. 1997). It should be noted that Ins+Dex-stimulated LPL activities in diabetic cultured cardiomyocytes were still significantly lower than hormone-stimulated enzyme activities in control cultured cells. The stimulation of HR-LPL activity in diabetic cardiomyocytes occurred at subnanomolar concentrations of the hormones (Fig. 3), suggesting that these hormones are relevant physiologically in the regulation of myocardial LPL activity in diabetic animals. Thus, although LPL activity was markedly reduced in diabetic cultured cardiomyocytes, the cells retained their responsiveness and sensitivity to Ins+Dex.

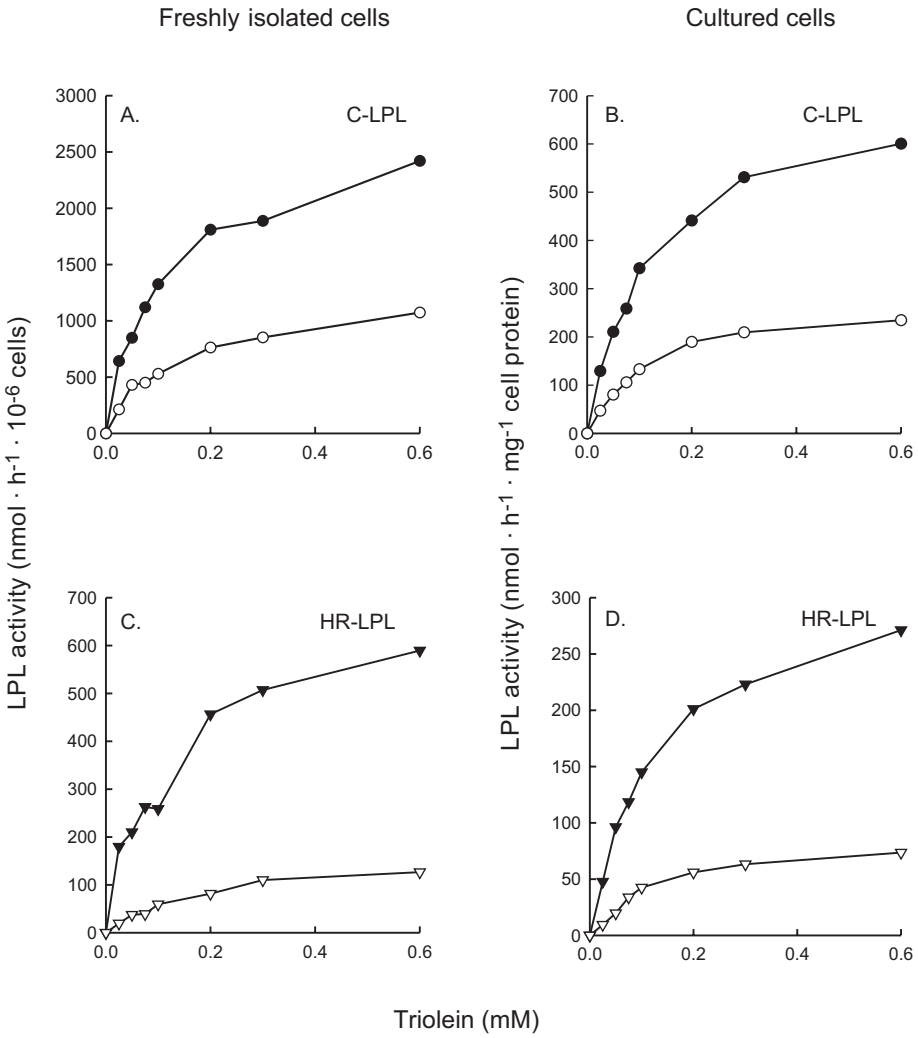
The time course for the stimulatory effect of Ins+Dex on HR-LPL activity in diabetic cultured cardiomyocytes was examined also (Table 1). Basal activity was quite constant over the range of culture times (3, 6, and 16 h). A significant increase in HR-LPL activity was observed after 3 h incubation with Ins+Dex and the maximal stimulatory effect was reached in 6 h. For experimental convenience, subsequent experiments utilized overnight (16 h) incubations, as in previous experiments with control cultured cells (Ewart et al. 1997).

In control cultured cardiomyocytes, the increase in C-LPL activity following Ins+Dex treatment was associated with an increase in LPL specific activity without a change in LPL protein mass (Ewart et al. 1997). Total LPL mass in diabetic cardiomyocytes cultured under basal conditions (90 ± 22 ng LPL protein/mg cell protein; $n = 6$) was not significantly higher than reported mass values in control cultured cells, which range from 29 (Ewart et al. 1997) to 64 ng LPL/mg cell protein (Ewart and Severson 1999). Ins+Dex treatment of diabetic cultured cardiomyocytes did not change LPL protein mass (86 ± 17 ng LPL/mg cell protein).

Cardiomyocytes from diabetic rats retained their phenotype of decreased LPL activity when cultured overnight in Joklik minimal essential medium (Fig. 1). This medium contains 11 mM glucose and no fatty acids, and therefore is not representative of in vivo conditions in a diabetic animal. Therefore, we examined whether increasing the glucose concentration or the presence of a fatty acid (oleic acid) in the culture medium would affect LPL activity in control and diabetic cardiomyocytes and (or) alter hormonal stimulation by Ins+Dex (Table 2). When glucose concentration in the culture medium was increased from 11 mM (NG) to 26 mM (HG) to approximate the hyperglycemia in streptozotocin-induced diabetic rats (Carroll et al. 1995), there was no effect on basal HR-LPL activities in control (245 and 205 nmol·h^{-1}·mg^{-1} cell protein, respectively, for NG and HG) or diabetic (110 and 107 nmol·h^{-1}·mg^{-1} cell protein, respectively) cardiomyocytes. In addition, the higher glucose concentration did not influence the inability of insulin alone or the ability of the Ins+Dex combination to stimulate HR-LPL activity in control and diabetic cultured cells (Table 2).

Addition of 60 μM oleic acid (OA; 2:1 molar ratio to albumin) to the overnight culture medium decreased basal HR-LPL activity in control cardiomyocytes from 245 to 93 nmol·h^{-1}·mg^{-1} cell protein (Table 2), as noted previously by Anderson et al. (1997). Previous control experiments have shown that the oleate-induced reduction in HR-LPL activity was not due to a

Fig. 2. Effect of varying substrate (triolein) concentration on LPL activities in freshly isolated and cultured cardiomyocytes. Radiolabelled triolein emulsions (specific activity 2 mCi/mmol) were prepared, and different aliquots were added to assay tubes to give a range of substrate concentrations (0.025 to 0.6 mM). LPL activity was measured in cellular lysates (C-LPL; Figs. 2A and 2B) and in post-heparin medium (HR-LPL; Figs. 2C and 2D) from freshly isolated (Figs. 2A and 2C) and cultured cardiomyocytes (Figs. 2B and 2D) from control (●, ▼) and diabetic (○, ▽) rat hearts. Similar results were obtained in a second independent experiment.

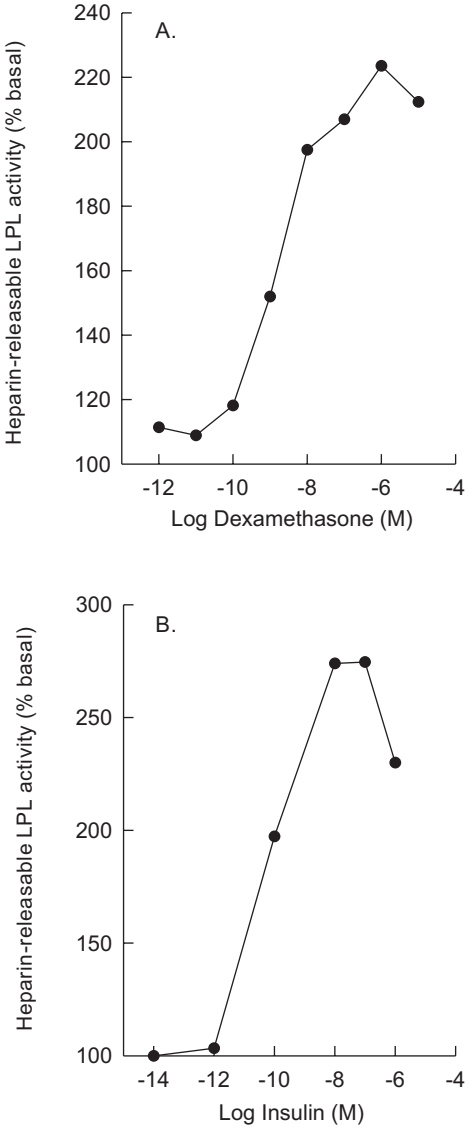


direct inhibitory effect on the LPL assay from the introduction of fatty acid from the culture medium (Anderson et al. 1997). Ins+Dex was still able to stimulate HR-LPL activity in control cells treated with oleate (to 296 nmol·h^{-1}·mg^{-1} cell protein); however, this hormonal treatment could not restore HR-LPL activity to control values (430 nmol·h^{-1}·mg^{-1} cell protein for control cells in NG with Ins+Dex). In diabetic cultured cardiomyocytes, the presence of oleic acid further inhibited the already reduced HR-LPL activity by 73% (from 110 to 30 nmol·h^{-1}·mg^{-1} cell protein; Table 2). Although Ins+Dex treatment stimulated HR-LPL activity by threefold in the presence of oleic acid (to 90 nmol·h^{-1}·mg^{-1} cell protein), this catalytic activity was significantly lower compared with hormone-stimulated HR-LPL activity in the absence of oleate (197 nmol·h^{-1}·mg^{-1} cell protein).

Discussion

The phenotype of reduced LPL activity observed in diabetic hearts and freshly isolated cells was retained following overnight culture (Figs. 1 and 2). This diabetes-induced change in myocardial LPL activity is, therefore, somewhat long lived and not readily reversed by overnight (16 h) incubation of the cells in culture medium, which does not reflect the metabolic substrate profile present in vivo during diabetes. There are only a few examples of myocardial enzyme activities altered in diabetes where the change persists during culture. The increased sensitivity of phosphorylase activation by epinephrine in freshly isolated cardiomyocytes from diabetic rat hearts (Wolleben et al. 1987) persisted after 3 h of culture, but this diabetes-induced hypersensitivity could no longer be observed after 24 h of culture because of marked changes in sensitivity of phosphorylase activation to epinephrine in control cardiomyocytes (Buczek-Thomas et al. 1992). The diabetes-induced reduction in glycogen synthase phosphatase activity in cardiomyocytes was still evident after 2 and 24 h of culture, but a profound decrease in glycogen synthase phosphatase activity in both control and diabetic cardiomyocytes during culture was a complicating factor (Jaspers et al. 1993). In contrast, basal HR-LPL activity in

Fig. 3. The concentration–response for insulin and dexamethasone in the stimulation of HR-LPL activity in cultured cardiomyocytes from diabetic rats. Cardiomyocytes from diabetic rats were incubated overnight with 100 nM insulin and the indicated concentrations of (A) dexamethasone or (B) 100 nM dexamethasone and the indicated concentrations of insulin. Each data point is the average activity determined from two separate culture wells; results are expressed as a percentage of basal HR-LPL activity (no additions).



diabetic cardiomyocytes following overnight culture was the same as activity measured 3 h after the initial cell attachment period (Table 1). Thus, the stimulatory action of Ins+Dex on LPL activity represents a real activation and not simply an inhibition of a culture-induced decrease in LPL activity. The stimulatory effect of Ins+Dex on LPL activity in diabetic cardiomyocytes occurs at low, physiological concentrations of the hormones (Fig. 3), as shown previously for control cardiomyocytes (Ewart et al. 1997), and neither hormone was effective alone. The response of LPL to hormonal stimulation in terms of sensitivity is, therefore, normal in diabetic cardiomyocytes. Although Ins+Dex-stimulated LPL activities in diabetic cultured cardiomyocytes were significantly lower than hormone-stimulated activities in control cultured cells, incubation of diabetic cardiomyocytes with Ins+Dex did restore C-LPL and HR-LPL activities to basal control levels. Since corticosterone is present in diabetic animals, this suggests that the absence of insulin per se is the direct cause of decreased myocardial LPL activity in type 1 diabetes.

In control cultured cardiomyocytes, the stimulation of C-LPL activity by Ins+Dex occurred without any change in LPL protein mass (Ewart et al. 1997; Ewart and Severson 1999); as a consequence, Ins+Dex increased LPL specific activity. This was somewhat unexpected since studies with adipocytes had shown the stimulatory effect of Ins+Dex on LPL activity was, at least in part, produced by increased LPL synthesis and (or) decreased degradation (Appel and Fried 1992; Oliver and Rogers 1993; Ottosson et al. 1994), mechanisms that would increase LPL mass. Newly synthesized LPL in the heart is inactive and monomeric; catalytic activity is acquired following processing of N-linked oligosaccharide chains in the endoplasmic reticulum and dimerization (Carroll et al. 1992; Liu et al. 1993). The simplest mechanism for the increased LPL specific activity in control cardiomyocytes cultured with Ins+Dex is increased conversion of an inactive monomeric LPL precursor to the active homodimeric enzyme (Ewart et al. 1997). A substantial pool of inactive LPL mass exists in control cultured cardiomyocytes (Ewart et al. 1997; Anderson et al. 1998), and previous investigations with adipocytes have shown that interconversion of inactive to active forms of LPL is a regulated process (Doolittle et al. 1990; Bergö et al. 1996). In diabetic cultured cardiomyocytes, the stimulation of C-LPL activity by Ins+Dex was not accompanied by a detectable change in LPL mass, suggesting that hormonal activation of LPL in diabetic cells also is due to increased enzyme specific activity. It must be acknowledged, however, that the presence of a large amount of inactive LPL mass in diabetic cardiomyocytes makes it very difficult to detect small changes that could potentially contribute to the increased catalytic activity. Future investigations measuring LPL synthesis and turnover from incorporation of [^{35}S]methionine into immunoprecipitable LPL protein in pulse and pulse–chase experiments (Carroll et al. 1995) will be necessary to conclude definitively that the Ins+Dex stimulation of C-LPL activity in diabetic cardiomyocytes is also because of an increase in enzyme specific activity (increased LPL catalytic activity without a corresponding increase in enzyme mass).

Insulin deficiency in diabetes leads to hyperglycemia and hyperlipidemia, which in turn may further impair energy metabolism. Diabetic cardiomyocytes retain low LPL activity in culture despite the absence of substrate conditions characteristic of the diabetic animal. Increasing the glucose concentration of the culture medium to 26 mM had no impact on either basal or hormone-stimulated HR-LPL activity in either control or diabetic cardiomyocytes (Table 2). Therefore, hyperglycemia is likely not a factor in regulating myocardial LPL activity in the diabetic rat, although Kern et al. (1987) have observed that basal constitutive release of LPL from human adipocytes was reduced when the glucose con-

Table 1. Stimulatory effect of insulin plus dexamethasone on heparin-releasable LPL activity at various time points.

	HR-LPL activity ($nmol \cdot h^{-1} \cdot mg^{-1}$ cell protein)		
Addition to culture medium	3 h (3)	6 h (5)	16 h (5)
None (basal)	170±16	199±18	176±25
Ins+Dex	275±52	370±39	328±56
%Stimulation	58±18	89±18	81±7

Note: HR-LPL activity was measured in diabetic cardiomyocytes cultured in the absence (basal) and in the presence of 100 nM insulin plus 100 nM dexamethasone (Ins+Dex) for 3, 6, and 16 h (overnight). Results are the mean ± SEM for the number of individual experiments in parentheses.

Table 2. The effect of glucose and oleic acid in the culture medium on HR-LPL activity in control and diabetic cardiomyocytes.

	HR-LPL activity ($nmol \cdot h^{-1} \cdot mg^{-1}$ cell protein)		
Addition to culture medium	NG	HG	OA
(A) Control cells (7)			
None (basal)	245±32	205±19	93±12†
Ins	293±49	267±30	154±12†
Ins+Dex	430±43	436±39	296±35†
(B) Diabetic cells (6)			
None (basal)	110±23	107±19	30±4†
Ins	139±23	145±17	42±9†
Ins+Dex	197±34	249±28	90±15†

Note: Cardiomyocytes from control or diabetic rats were cultured overnight in normal medium (11 mM glucose; NG), high glucose (26 mM; HG) medium, or normal medium containing oleic acid (60 μM; OA). These incubations also contained no further additions (basal), 100 nM insulin (Ins), or 100 nM insulin plus 100 nM dexamethasone (Ins+Dex). Results are the mean ± SEM of six and seven separate experiments for diabetic and control groups, respectively. Data were analysed using one-way ANOVA. Comparisons were made using Dunnett's multiple comparisons test ($p < 0.05$).

†Significantly different from the corresponding incubation in normal (NG) medium.

centration in the culture medium was increased to 25 mM. The inclusion of oleic acid in the culture medium of control cardiomyocytes leads to a marked reduction in basal HR-LPL activity (Anderson et al. 1997). Incubation of control cells with fatty acids may thus represent a means of producing the diabetic phenotype of low LPL activity in vitro; fatty acids also inhibit LPL activity in cultured adipocytes (Amri et al. 1996). In diabetic cardiomyocytes, oleic acid further decreased the already reduced basal HR-LPL activity and thus reduced the absolute activity obtained in the presence of Ins+Dex (Table 2). It is clear, however, that Ins+Dex stimulation of LPL in cultured cardiomyocytes was not prevented by oleic acid; rather, the primary effect of the fatty acid was to reduce basal LPL activities. This mechanism is very different from reports of hormonal resistance produced by fatty acids. For example, the presence of fatty acids in the culture medium of cardiomyocytes reduced stimulation of glucose transport and glycogen synthase by insulin (Jaspers et al. 1993; Eckel et al. 1991). Interestingly, fatty acids also suppressed the ability of glucocorticoids to induce urea cycle enzymes in cultured hepatocytes (Tomomura et al. 1996).

In summary, the diabetes-induced reduction in LPL activity in cardiomyocytes was still evident after overnight culture. The combination of Ins and Dex was able to stimulate C-LPL activity in cultured diabetic cardiomyocytes, possibly as a result of an increased enzyme specific activity. The mechanism of this stimulatory action of Ins+Dex on LPL specific activity in cultured control and diabetic cardiomyocytes is currently under investigation. Regulation of myocardial LPL activity in diabetes probably involves a complex interplay of hormones and metabolic factors.

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