Insulin and dexamethasone stimulation of cardiac lipoprotein lipase activity involves the actin-based cytoskeleton

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Lipoprotein lipase (LPL) activity in cultured ventricular cardiomyocytes from adult rat hearts was stimulated by the combination of insulin (100 nM) and dexamethasone (100 nM) during an overnight (16 h) incubation. Wortmannin (100 nM), rapamycin (30 ng/ml) or PD98059 (50 μ M) did not prevent this stimulation, suggesting that phosphatidylinositol 3-kinase, p70 S6 kinase and the mitogen-activated protein kinase cascade are not involved in transducing the hormonal signal. In contrast, cytochalasin D (2 μ M) completely abolished the stimulatory

effect of insulin and dexamethasone on both heparin-releasable LPL and total cellular LPL activities. The potential role of the actin cytoskeleton in the stimulation of LPL activity by insulin and dexamethasone appears to be distal to the initial signalling events since cytochalasin D is still effective in preventing the stimulation when added 2 h after the hormones.

Key words: glucocorticoid, heart, lipoprotein metabolism.

INTRODUCTION

Lipoprotein lipase (LPL; EC 3.1.1.34) catalyses the hydrolysis of triacylglycerol to 2-monoacylglycerol and fatty acids. LPL is synthesized as an inactive monomer ($\approx$ 55 kDa) that contains N-linked oligosaccharides [1,2]. After trimming of terminal glucose residues by glucosidases in the endoplasmic reticulum, LPL oligomerizes to form catalytically active homodimers [3–6]. The oligosaccharide chains of LPL undergo further Golgi processing to mature, endoglycosidase-H-resistant forms, and LPL is then translocated to the plasma membrane for secretion as a glycoprotein. In heart [3,7,8], LPL is synthesized and processed in cardiomyocytes and is then exported to the coronary vasculature where, bound to heparan-sulphate proteoglycans on the endothelial surface, the functional enzyme acts in the clearance of triacylglycerol-rich lipoproteins, chylomicrons and very-low-density lipoproteins. The fatty acids released by LPL action are taken up by cardiomyocytes to fuel oxidative metabolism.

Cardiac LPL activity (endothelium-bound and in isolated cardiomyocytes) is depressed after induction of insulin-dependent diabetes [9–12] and can be normalized by administration of insulin to diabetic rats [9–11,13]. Insulin treatment of control animals also stimulated cardiac LPL activity [10,11]. However, insulin does not stimulate LPL when added to incubations *in vitro* of cardiomyocytes from control or diabetic hearts [11], suggesting the requirement for an additional factor *in vivo*. We showed previously that the combination of a glucocorticoid (dexamethasone) with insulin resulted in a stimulation of LPL activity in cultured cardiomyocytes [14]. This occurred through a post-transcriptional and post-translational mechanism, since LPL protein mass (determined by ELISA) was unchanged; as a result, LPL-specific catalytic activity was increased. Since neither insulin nor dexamethasone were effective in eliciting the stimulation alone, the action of the hormones may be regarded as permissive. The signalling mechanism(s) underlying this stimulatory effect on LPL activity is (are) unknown. Therefore, we have examined the potential involvement of key insulin-signalling components [phosphatidylinositol 3-kinase (PI 3-kinase), p70 S6

kinase and mitogen-activated protein kinase (MAP kinase)] [15,16] in eliciting the stimulation of LPL activity in cultured cardiomyocytes by insulin and dexamethasone. PI 3-kinase activation mediates many of the acute metabolic actions of insulin such as increasing glucose transport; p70 S6 kinase is downstream of PI 3-kinase and regulates protein synthesis, whereas stimulation of the MAP-kinase pathway can change myocardial gene expression. In addition, we have examined the potential involvement of the actin-based cytoskeleton since both insulin [17] and glucocorticoids [18] are reported to cause cytoskeletal rearrangements that are integral to some of their actions.

EXPERIMENTAL

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/dark cycle and allowed access to tap water and standard laboratory chow *ad libitum*. All procedures involving animals were approved by the Committee on Animal Bioethics and Care at the University of Calgary.

Materials

Joklik minimal essential medium and penicillin/streptomycin were purchased from Gibco Canada (Burlington, ON, Canada). Collagenase was purchased from Technicon Canada Ltd. (Richmond, BC, Canada). Heparin (1000 units/ml; Hepalean) was obtained from Organon Teknika (Toronto, ON, Canada). Laminin was from Becton Dickinson Labware (Bedford, MA, U.S.A.). Insulin, dexamethasone, wortmannin, cytochalasin D and essentially fatty acid-free BSA were products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). Rapamycin, PD98059, and LY294002 were products of Calbiochem (La Jolla, CA,

Abbreviations used: LPL, lipoprotein lipase; PI 3-kinase, phosphatidylinositol 3-kinase; Ins/Dex, 100 nM insulin plus 100 nM dexamethasone; HR-LPL, heparin-releasable LPL; C-LPL, cellular LPL; MAP kinase, mitogen-activated protein kinase.

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U.S.A.). Latrunculin B was from Biomol (Plymouth Meeting, PA, U.S.A.). Glycerol-[9,10-³H]trioleate was from Amersham Canada (Oakville, ON, Canada). Western-blot analysis of the phosphorylation status of p44 and p42 MAP kinases was performed using a MAP-kinase antibody kit obtained from New England BioLabs (Mississauga, ON, Canada).

Preparation and incubation of cardiomyocytes

Ventricular cardiomyocytes from adult rat hearts were isolated under aseptic conditions by collagenase treatment as described previously [14,19]. Freshly isolated cells were suspended in culture medium [Joklik minimal essential medium supplemented with 25 mM NaHCO₃/1 mM CaCl₂/1.2 mM MgSO₄/1 mM D,L-carnitine/100 IU/ml penicillin/100 µg/ml streptomycin/0.2% (w/v) BSA (pH 7.4)] that 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 haemocytometer. A preparation from a single heart yielded (7–9) × 10⁶ viable cells that were diluted to a density of 150 000 cells/ml.

Cardiomyocytes were cultured in laminin-coated six-well (35 mm) plates as described previously [14]. After 3 h, the cells were provided with fresh culture medium without (basal conditions) or with 100 nM insulin plus 100 nM dexamethasone (Ins/Dex). When the effects of wortmannin (100 nM), LY294002 (30 µM), rapamycin (30 ng/ml) or PD98059 (50 µM) were investigated, these compounds were added 30 min prior to the addition of the hormones and were present either throughout the overnight incubation (wortmannin, LY 294002, rapamycin) or for up to 6 h (PD98059). Cytochalasin D (2 µM) was added at the same time as the hormones or 2 or 6 h after hormone addition. Cells were routinely incubated overnight (16 h) at 37 °C under a humidified atmosphere of 95% O₂/5% CO₂. The cells were then treated with fresh culture medium (1 ml/well) with or without 5 units/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 (C-LPL) that is bound to the cell surface of cardiomyocytes [10]. Cardiomyocytes incubated in the absence of heparin were scraped into lysis buffer [50 mM ammonia buffer (pH 8.0) containing 0.05% Triton X-100], sonicated and assayed for total C-LPL activity.

Assay of LPL activities

LPL activities were determined by measuring hydrolysis of a sonicated [³H]triolein substrate emulsion [19], except that the specific activity of [³H]triolein was increased by decreasing the total triolein concentration in the assay from 0.6 to 0.1 mM. Thus the assay contained 0.1 mM glycerol-[9,10-³H]trioleate (6 mCi/mmol), 25 mM Pipes (pH 7.5), 0.05% (w/v) essentially fatty acid-free BSA, 50 mM MgCl₂ and 2% heat-inactivated chicken serum as the LPL activator. For assay of cell lysates, 2 units/ml heparin were 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; [³H]oleate generated by the action of LPL was measured by liquid–liquid extraction [19] and radioactive scintillation counting. All assays were performed in duplicate; LPL activity is expressed as nmol of oleate released/h per mg of protein in the sonicated cell extracts. Protein concentration was measured using a Coomassie Brilliant Blue spectrophotometric assay [20] with BSA as standard.

ELISA for LPL

The ELISA for LPL was performed as described previously [14]. Samples were prepared by lysing cells from 10-cm dishes in 0.2 ml of 25 mM NH₄Cl/5 mM EDTA/0.8% (w/v) Triton X-100/0.04% (w/v) SDS/33 µg/ml heparin/10 µg/ml leupeptin (pH 8.8). Dishes were washed once with 0.3 ml of buffer containing 250 mM sucrose, 1 mM EDTA, 1 mM dithiothreitol and 20 mM Hepes (pH 7.4) and this wash was combined with the lysate and sonicated. LPL mass is expressed as ng/mg of cell protein; LPL-specific activity is expressed as m-units/ng of LPL protein, where 1 m-unit is defined as the amount of enzyme catalysing the release of 1 nmol of oleate/min.

Immunoblotting

Cardiomyocytes in six-well plates were treated with or without PD98059 (50 µM) for 6 h or overnight (16 h) and then stimulated with insulin (100 nM) or PMA (100 nM) for 10 min. Cells were washed once in cold PBS and lysed in buffer [1% Triton X-100/10 mM Tris/HCl (pH 7.5)/1 mM EDTA/1 µM pepstatin A/1 µM leupeptin/200 µM PMSF/40 mM NaF/7.5 mM sodium pyrophosphate/1.5 mM Na₃VO₄]. Lysates (30 µg of cell protein) were subjected to SDS/PAGE and transferred on to nitrocellulose membranes. Membranes were probed with antisera containing polyclonal antibodies that recognize either total p44/p42 MAP kinase (1:1000 dilution) or tyrosine-204-phosphorylated MAP kinase (1:1000 dilution) followed by anti-rabbit IgG conjugated to horseradish peroxidase (1:2000 dilution). Proteins were visualized by the enhanced chemiluminescence method.

Statistics

Results are expressed as means ± S.E.M., where *n* is the number of individual cultured cardiomyocyte preparations. Comparisons were made using Student's unpaired or paired *t* test or one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple-comparisons test, with statistical significance corresponding to *P* < 0.05.

RESULTS

Overnight treatment of cardiomyocytes with Ins/Dex stimulated both C-LPL (149% of basal levels) and HR-LPL (174% of basal levels) activities (Table 1), as reported previously [14]. To investigate the potential insulin-signalling mechanism involved in the hormonal activation of LPL, incubations were performed in the presence of wortmannin, rapamycin or PD98059, agents that inhibit PI 3-kinase, p70 S6 kinase and MAP kinase kinase, respectively [15]. Wortmannin and rapamycin had no effect on basal C-LPL and HR-LPL activities, nor did they block the stimulatory effect of Ins/Dex on LPL activity. Wortmannin (100 nM) completely inhibited insulin-stimulated 2-deoxyglucose uptake in cultured cardiomyocytes (results not shown), indicating that PI 3-kinase activity was inhibited. Since wortmannin may be degraded rapidly by some cell types [21], we also performed experiments using a 10-fold higher concentration (1 µM) of wortmannin or instead added LY294002 (30 µM), another compound that inhibits PI 3-kinase. In each case, basal and hormone-stimulated LPL activities were not affected (results not shown). The effectiveness of PD98059 (50 µM) as a MAP kinase kinase inhibitor was lost during the overnight culture since insulin

Table 1 The effect of wortmannin and rapamycin on C-LPL and HR-LPL activities in cardiomyocytes incubated overnight without or with Ins/Dex

Cardiomyocytes were cultured overnight with no additions (basal) or with Ins/Dex. When present, wortmannin (100 nM) or rapamycin (30 ng/ml) was added 30 min prior to the hormones and were present throughout the subsequent overnight (16 h) incubation. Results are the means $\pm$ S.E.M. of four individual experiments for C-LPL and HR-LPL activities (no-addition and wortmannin experiments) or the average of two separate experiments (rapamycin experiment), and are presented as a percentage of basal activities (C-LPL, 100% = 461 ± 91 nmol/h per mg of cell protein; HR-LPL, 100% = 291 ± 66 nmol/h per mg of cell protein).

Additions	LPL activity (% of basal)			
	C-LPL		HR-LPL	
	Basal	Ins/Dex	Basal	Ins/Dex
None	100	149 $\pm$ 17	100	174 $\pm$ 11
Wortmannin	97 $\pm$ 4	147 $\pm$ 15	102 $\pm$ 9	168 $\pm$ 25
Rapamycin	99	169	95	225

Table 2 The effect of cytochalasin D on C-LPL and HR-LPL activities in cardiomyocytes incubated without or with Ins/Dex

Cardiomyocytes were incubated overnight with no additions (basal) or with Ins/Dex. Cytochalasin D (2 μ M) was added at the same time as the hormones. Results are the means $\pm$ S.E.M. of four individual experiments for C-LPL (100% = 364 ± 92 nmol/h per mg of cell protein) and HR-LPL (100% = 160 ± 47 nmol/h per mg of cell protein) activities. Comparisons were made using Student's *t* test ($P < 0.05$).

Additions	LPL activity (% of basal)			
	C-LPL		HR-LPL	
	Basal	Ins/Dex	Basal	Ins/Dex
None	100	189 $\pm$ 16*	100	241 $\pm$ 20*
Cytochalasin D	82 $\pm$ 18	88 $\pm$ 13	79 $\pm$ 7†	93 $\pm$ 6

* Significantly different from the corresponding basal activity.

† Significantly different from the activity in the absence of cytochalasin D.

treatment the following morning still stimulated MAP kinase (results not shown). However, insulin- or PMA-stimulated MAP kinase activity was inhibited following a 6-h preincubation with PD98059 (results not shown), which is within the time frame of signal transduction and development of LPL stimulation by Ins/Dex treatment. The presence of 50 μ M PD98059 with hormones for 6 h did not reduce Ins/Dex stimulation of HR-LPL activity.

A number of hormone-stimulated processes require an intact actin cytoskeleton [22]. Cytochalasin D disrupts actin filaments and inhibits actin polymerization [23]. Therefore, to investigate the possible involvement of the actin cytoskeleton in LPL activity in cultured cardiomyocytes, cytochalasin D was added to overnight incubations. Cytochalasin D (2 μ M) resulted in a small but significant decrease in basal HR-LPL activity (Table 2). More remarkably, the presence of cytochalasin D in the overnight culture medium completely abolished the stimulatory effect of Ins/Dex on both C-LPL and HR-LPL activities. The inhibitory effect of cytochalasin D was maximal at a concentration of 1 μ M (Figure 1). We also performed some experiments with latrunculin B (2 μ M), a structurally unrelated compound that also disassembles the actin cytoskeleton [24]. Latrunculin B did not appreciably affect basal HR-LPL activity (109 ± 20 versus 125 ± 16 nmol/h per mg of cell protein with and without latrunculin B, respectively), whereas it significantly reduced Ins/Dex-stimulated HR-LPL activity (to 191 ± 7 from 270 ± 16 nmol/h per mg of cell protein, $n = 3$, $P < 0.05$), again implicating the actin-cytoskeletal network in eliciting the hormonal stimulation of cardiac LPL activity.

Ins/Dex treatment does not influence the amount of total LPL protein in cardiomyocytes [14]. The possibility that cytochalasin D reduced LPL protein was investigated by measuring LPL mass by the ELISA technique. Cytochalasin D did not significantly alter LPL mass in hormone-treated cardiomyocytes (Table 3). Consequently, enzyme-specific activity, calculated by dividing LPL activity by the corresponding mass value, was decreased. Therefore, the normal stimulatory effect of Ins/Dex treatment on LPL-specific activity was not offset by a reduction in LPL mass in the presence of cytochalasin D.

Cytochalasin D could potentially interfere with the initial hormone-signalling steps [22], distal steps elicited by the signalling cascade, or both. To examine these possibilities (Table 4), experiments were conducted in which hormones were incubated with cultured cardiomyocytes for only 2 or 6 h in addition to the routine 16 h (overnight) incubation. In the case of the 16 h incubation, cytochalasin D (2 μ M) was added 2 or 6 h after addition of Ins/Dex. When cytochalasin D was added 2 h after Ins/Dex, the stimulatory effect of Ins/Dex on HR-LPL activity after overnight culture was still blocked; a 2-h incubation with Ins/Dex was insufficient to stimulate HR-LPL activity (Table 4). Although a 6-h incubation with Ins/Dex did produce a near-maximal stimulatory effect on HR-LPL activity, addition of cytochalasin D after a 6-h incubation with hormones still reduced HR-LPL activity to the control (basal) level. These results illustrate two important aspects of the inhibitory effect of cytochalasin D. First, although cytochalasin D is known to interfere with insulin signalling [22], its inhibition of LPL activation by Ins/Dex treatment appears to be on a step (or

Figure 1 Concentration response for cytochalasin D inhibition of HR-LPL activity in cardiomyocytes incubated overnight with Ins/Dex

Cardiomyocytes were incubated overnight with Ins/Dex and the indicated concentrations of cytochalasin D. Each data point is the mean HR-LPL activity determined from duplicate culture wells and is presented as a percentage of basal activity (no hormone additions; 100% = 424 nmol/h per mg of cell protein). A second experiment gave similar results.

steps) distal to initial signalling events, since it is effective when added 2 h after the addition of the hormones. Second, since LPL activation was near maximal after 6 h of incubation with Ins/Dex, the effectiveness of cytochalasin D, even when added at this time point, shows that it not only inhibits the development of LPL activation but will also reverse an already established activation during the subsequent overnight incubation.

DISCUSSION

We have reported previously that incubation of cultured cardiomyocytes from adult rat hearts with Ins/Dex stimulated both C-LPL and HR-LPL activities [14] but that the signalling mechanism whereby the hormones elicit this response is unknown. We report here (Table 1) that the stimulatory effect of Ins/Dex on LPL activity in cultured cardiomyocytes is not blocked by inhibitors of PI 3-kinase (wortmannin and LY294002), p70 S6 kinase (rapamycin) or the MAP-kinase pathway (PD98059). Therefore, activation of insulin-signalling pathways involving these enzymes must not be required for the activation of LPL in cultured cardiomyocytes by the Ins/Dex combination. This result is different from recent observations of Kraemer et al. [25], who reported that stimulation of adipose-tissue LPL activity by insulin involves both wortmannin- and rapamycin-sensitive pathways. In their experiments with rat adipocytes, wortmannin (1 μM) completely blocked the stimulatory effect of insulin on HR-LPL activity observed after a 4-h incubation; rapamycin produced a partial (60%) inhibition of insulin-stimulated LPL activity [25]. There are, however, a number of marked differences

Table 3 Effect of cytochalasin D on C-LPL protein mass and specific activity

Cardiomyocytes were cultured in the absence (basal) or presence of Ins/Dex or Ins/Dex plus 2 μM cytochalasin D (Cyto D). Results are the means ± S.E.M. of four separate experiments. Comparisons were made using the Student–Newman–Keuls multiple-comparisons test ($P < 0.05$).

Additions	Catalytic activity (m-units/mg of protein)	Mass (ng of LPL/mg of protein)	Specific activity (m-units/ng of LPL)
None (basal)	6.12 ± 1.27	64.4 ± 6.3	0.09 ± 0.02
Ins/Dex	9.62 ± 1.99*	71.4 ± 2.5	0.14 ± 0.03*
Ins/Dex + Cyto D	4.97 ± 0.81†	60.9 ± 3.7	0.08 ± 0.01†

* Significantly different from the basal activity.

† Significantly different from the activity in the presence of Ins/Dex alone.

Table 4 The effect of cytochalasin D on HR-LPL activity in cardiomyocytes first stimulated with Ins/Dex

Cardiomyocytes were incubated with Ins/Dex for 2 h, 6 h or overnight (16 h) and the HR-LPL activity determined. In some overnight incubations cytochalasin D (2 μM) was added 2 or 6 h following the addition of the hormones and HR-LPL activity was determined following overnight incubation. Results are the means ± S.E.M. of six separate experiments. Comparisons were made using the Student–Newman–Keuls multiple-comparisons test ($P < 0.05$).

Cytochalasin D addition	Total culture time (h)	HR-LPL activity (nmol/h per mg of cell protein)	
		Basal	Ins/Dex
None	2	98.7 ± 11.0	104.4 ± 9.2
None	6	126.8 ± 15.0	201.4 ± 24.1*
None	16	137.5 ± 12.5	259.6 ± 19.9*
After 2 h	16		139.0 ± 18.7
After 6 h	16		150.9 ± 29.1†

* Significantly different from the corresponding basal level.

† Significantly different from the activity after 6 h of Ins/Dex treatment.

between the hormonal regulation of LPL in adipose and cardiac tissues. Most notably, insulin (in the absence of glucocorticoids) directly stimulates LPL activity in adipose tissue through complex biochemical mechanisms that include increases in LPL mRNA levels and LPL synthesis [26,27] along with post-translational mechanisms [28]. Furthermore, the potentiation of insulin activation of adipose-tissue LPL by glucocorticoids was the result of increased LPL synthesis [29] and/or decreased LPL degradation [30]. Thus changes in LPL mass dominate mechanisms that are responsible for the stimulation of LPL activity in adipose tissue by insulin alone and the combination of insulin and glucocorticoids. In contrast, insulin alone has no direct stimulatory effect on LPL activity in cardiomyocytes, and the stimulation of LPL activity by the combination of Ins/Dex occurs without a detectable increase in LPL protein mass [14]. It is not surprising, therefore, that the regulation of cardiac LPL in terms of sensitivity to wortmannin and rapamycin is different from that in adipose tissue [25].

Experiments with cytochalasin D revealed intriguing results which implicate the actin cytoskeleton in the hormonal regulation of LPL in cultured cardiomyocytes. Visualization of the cytoskeletal actin network in cardiomyocytes with techniques such as staining with rhodamine-conjugated phalloidin is complicated by the extensive actin content in the sarcomeric contractile apparatus [31,32]. Consequently, documentation of disaggregating effects of cytochalasin D (2 μ M) on the non-sarcomeric actin cytoskeleton in adult cardiomyocytes has been restricted to observations after prolonged cell culture in the presence of serum, which results in loss of the organized structure of myofibrillar proteins [33]. The present experiments have all been conducted with adult cardiomyocytes maintained in short-term culture in the absence of serum to minimize phenotypic changes in morphology. It might have been anticipated that long-term overnight (16 h) incubations with cytochalasin D and the attendant disruption of the actin-based cytoskeleton in cardiomyocytes would have markedly reduced basal HR-LPL activity, since heparan-sulphate-proteoglycan-binding sites for LPL on the cell surface of fibroblasts are associated with the actin cytoskeleton [34]. As a consequence, 1 μ M cytochalasin D produced a disorganization of LPL-binding sites at the fibroblast cell surface. However, cytochalasin D produced only a modest reduction in basal HR-LPL activity (Table 2) and no change in total C-LPL activity. Disorganization of LPL-binding sites on the cardiomyocyte cell surface could have resulted in an accumulation of active intracellular LPL but this was not observed. Similarly, latrunculin B had no effect on basal HR-LPL activity. Therefore, the presumed disruption of the actin cytoskeleton in cardiomyocytes with cytochalasin D does not appear to alter significantly the amount of surface-bound enzyme that can be displaced into the medium with heparin (HR-LPL activity) under basal conditions.

Cytochalasin D treatment of cultured cardiomyocytes resulted in the complete inhibition of Ins/Dex-stimulated LPL activity (Tables 2 and 3). It is important to emphasize that the Ins/Dex stimulation of C-LPL activity, which includes both the component of LPL activity on the cell surface that is displaceable by heparin as well as 'residual' heparin-non-releasable intracellular activity [14], was blocked completely by cytochalasin D, along with HR-LPL activity. This effect was not due to a reduction in LPL mass induced by cytochalasin D, but a decrease in enzyme-specific activity to a level not different from that in basally treated cardiomyocytes (Table 3). Since it is the Ins/Dex-stimulated LPL activity that is principally affected by cytochalasin D, it is possible that cytoskeletal disruption prevents a required hormone-signalling event. The cytoskeleton has been

implicated in the signalling of both insulin [17] and glucocorticoids [18]. However it is clear from Table 4 that the cytochalasin D-sensitive step is not at the level of early signalling steps, since cytochalasin D added 2 h after Ins/Dex still inhibited the stimulation of HR-LPL activity. The results suggest that a more distal step, leading to the hormone-induced increase in LPL-specific enzyme activity, is the site of cytochalasin D inhibition.

It is difficult to identify a site of action for cytochalasin D that prevents the hormonal stimulation of LPL activity in cardiomyocytes because the molecular mechanism whereby Ins/Dex treatment increases LPL-specific activity without a change in LPL mass (Table 3; [14]) is unknown. An alteration in the ratio of active (dimeric) to inactive (monomeric) forms of LPL in cardiomyocytes seems the most likely mechanism for changes in LPL-specific activity, as shown for the fasting-induced fall in adipose-tissue LPL activity [35]. The acquisition of LPL catalytic activity occurs in the endoplasmic reticulum as the result of N-linked glycosylation, glucose trimming and dimerization [1,3,4], although there is some evidence that an extra-endoplasmic reticulum event may also be required [36]. Ins/Dex could influence processing/dimerization of LPL or, alternatively, the hormones might alter the catalytic efficiency of the enzyme by an unknown mechanism or stabilize catalytically active LPL by inhibiting an inactivation process. Any of these steps are potential sites for regulation by Ins/Dex; therefore, a challenge for future research will be to identify which process(es) is (are) dependent on the actin cytoskeleton. If cytochalasin D were able to interfere with LPL processing, a pronounced inhibitory effect of cytochalasin D on basal activity would be expected. An Ins/Dex-induced change in LPL protein stability (i.e. an inactivation process dependent on the actin cytoskeleton) would, however, be consistent with our observation that cytochalasin D added 6 h after Ins/Dex effectively reversed what would have been an already established Ins/Dex activation of LPL (see Table 4).

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