Acute dexamethasone-induced increase in cardiac lipoprotein lipase requires activation of both Akt and stress kinases

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Kewalramani G, Puthanveetil P, Kim MS, Wang F, Lee V, Hau N, Beheshti E, Ng N, Abrahani A, Rodrigues B. Acute dexamethasone-induced increase in cardiac lipoprotein lipase requires activation of both Akt and stress kinases. Am J Physiol Endocrinol Metab 295: E137–E147, 2008. First published May 6, 2008; doi:10.1152/ajpendo.00004.2008.—Following dexamethasone (DEX), cardiac energy generation is mainly through utilization of fatty acids (FA), with DEX animals demonstrating an increase in coronary lipoprotein lipase (LPL), an enzyme that hydrolyzes lipoproteins to FA. We examined the mechanisms by which DEX augments cardiac LPL. DEX was injected in rats, and hearts were removed, or isolated cardiomyocytes were incubated with DEX (0–8 h), for measurement of LPL activity and Western blotting. Acute DEX induced whole body insulin resistance, likely an outcome of a decrease in insulin signaling in skeletal muscle, but not cardiac tissue. The increase in luminal LPL activity after DEX was preceded by rapid nongenomic alterations, which included phosphorylation of AMPK and p38 MAPK, that led to phosphorylation of heat shock protein (HSP)25 and actin cytoskeleton rearrangement, facilitating LPL translocation to the myocyte cell surface. Unlike its effects in vivo, although DEX activated AMPK and p38 MAPK in cardiomyocytes, there was no phosphorylation of HSP25, nor was there any evidence of F-actin polymerization or an augmentation of LPL activity up to 8 h after DEX. Combining DEX with insulin appreciably enhanced cardiomyocyte LPL activity, which closely mirrored a robust elevation in phosphorylation of HSP25 and F-actin polymerization. Silencing of p38 MAPK, inhibition of PI 3-kinase, or preincubation with cytochalasin D prevented the increases in LPL activity. Our data suggest that, following DEX, it is a novel, rapid, nongenomic phosphorylation of stress kinases that, together with insulin, facilitates LPL translocation to the myocyte cell surface.

heart; adenosine monophosphate-activated protein kinase; actin cytoskeleton; fatty acids

GLUCOCORTICOIDS ARE USED WIDELY as anti-inflammatory and immunosuppressive agents (42). However, chronic glucocorticoid therapy is often associated with adverse effects, including Cushings’s syndrome, osteoporosis, gastrointestinal bleeding, and dyslipidemia (42). In addition, both excess endogenous (32) and exogenous (43) glucocorticoids contribute toward the generation of the metabolic syndrome, including obesity, hypertension, and insulin resistance. In the human body, insulin resistance is viewed as an insufficiency in insulin action, which can lead to a cardiac pathology. In the San Antonio Heart Study, patients with insulin resistance had a 2.5 times increased risk of dying of cardiovascular disease than those without insulin resistance (20). Additionally, patients who have insulin resistance can develop type 2 diabetes. Diabetes itself promotes vascular diseases and nonvascular cardiac injury (2). With insulin resistance or diabetes, metabolism in multiple organ systems including the heart is altered, which is believed to be an important factor in increased morbidity and mortality (27). Compared with glucose, fatty acids (FA) are the preferred substrate consumed by cardiac tissue (31), with hydrolysis of triglyceride (TG)-rich lipoproteins by lipoprotein lipase (LPL) positioned at the endothelial surface of the coronary lumen being suggested to be the principal source of FA for cardiac utilization (3). Increasing FA uptake through overexpression of cardiac human LPL (47) or fatty acid transport protein (9), or augmenting FA oxidation through overexpression of cardiac peroxisome proliferator-activated receptor-α (17) or long-chain acyl-CoA synthase (10), results in a severe cardiac pathology.

Glucocorticoids act through genomic and nongenomic pathways (6). Genomic mechanisms include binding of the glucocorticoid to its cytosolic receptor, relocation into the nucleus, and an increase or decrease in gene expression (6). Nongenomic modes of glucocorticoid action occur rapidly (few seconds to minutes), and include activation of numerous cellular processes like mitogen-activated protein kinases (MAPKs) and heterotrimeric GTP-binding proteins (G proteins) (8). Through its nongenomic effects in uncoupling oxidative phosphorylation, glucocorticoids decrease ATP in cells within the immune system, leading to cell death, thereby preventing acute immune responses (7). If this effect of glucocorticoids were mimicked in cardiac tissue, the heart would be expected to change its substrate utilization in an effort to maintain its levels of ATP. Indeed, using an acute dexamethasone (DEX) model, our laboratory has reported that glucocorticoid treatment leads to amplification in coronary LPL (both LPL activity and LPL protein) (37). The resulting clearance of plasma TG increases FA delivery to the heart, augments FA (but decreases glucose) oxidation (37, 36), and ensures continuous ATP generation to maintain normal heart function. Under these conditions, an increase in oxygen demand and generation of reactive oxygen species could lead to the cardiac damage seen following chronic glucocorticoid use.

In the heart, as endothelial cells do not express the LPL gene, this enzyme is synthesized in myocytes and subsequently transported onto heparan sulfate proteoglycan (HSPG) binding sites on the myocyte cell surface (14). From there, the enzyme...
GLUCOCORTICOID EFFECTS ON HEART METABOLISM

Table 1. General characteristics of experimental animals

<table>
<thead>
<tr>
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<th>CON</th>
<th>DEX</th>
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<tr>
<td>Body weight, g</td>
<td>265±4</td>
<td>273±4</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.5±0.1</td>
<td>1.3±0.05</td>
</tr>
<tr>
<td>GIR, mg·kg⁻¹·min⁻¹</td>
<td>15±2.2</td>
<td>4.0±0.7*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>8.2±0.2</td>
<td>8.0±0.4</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>3.0±0.01</td>
<td>3.0±0.05</td>
</tr>
<tr>
<td>Plasma FA, mmol/l</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Plasma TG, mmol/l</td>
<td>1.5±0.1</td>
<td>0.5±0.1*</td>
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Values are means ± SE; n = 3–5 rats in each group. CON, control; DEX, dexamethasone; GIR, glucose infusion rate; FA, fatty acids; TG, triglyceride. Shown are general characteristics of experimental animals after DEX (1 mg/kg) was administered by ip injection into CON rats, and animals were killed after 4 h. Whole animal insulin resistance was also assessed using a euglycemic hyperinsulinemic clamp. Briefly, 4 h after injection of vehicle or DEX, animals were anesthetized with pentobarbital sodium (Somnotol; 65 mg/kg), and a cannula was inserted into the left jugular vein. Insulin (Humulin R) was administered by ip injection into CON rats, and animals were killed after 4 h. Whole animal insulin resistance was also assessed using a euglycemic hyperinsulinemic clamp, a direct measure of insulin sensitivity, this dose of DEX induces whole body insulin resistance (37). Subsequently, hearts were removed for measurement of coronary luminal LPL activity and Western blotting.

Euglycemic hyperinsulinemic clamp. Whole animal insulin resistance was assessed using a euglycemic hyperinsulinemic clamp, as described previously (37).

MATERIALS AND METHODS

Experimental animals. The investigation conformed to the guide for the care and use of laboratory animals published by the US National Institutes of Health and the University of British Columbia (UBC). Adult male Wistar rats (260–300 g) were obtained from the UBC Animal Care Unit. The synthetic glucocorticoid hormone DEX (1 mg/kg) or an equivalent volume of ethanol was administered by intraperitoneal injection to nonfasted rats, and the animals were euthanized at various times (0–4 h; plasma half-life of DEX is ~279 min). In the human body, the basal daily secretion of cortisol is ~6–8 mg/m² (in a 70-kg adult male this translates to ~0.2 mg/kg). In response to stress, cortisol release is increased up to 10-fold of the basal value (2 mg/kg). For exogenous administration, the dosing with corticosteroids depends on the disease condition and varies from 75 to 300 mg/day (~1–4 mg/kg). Previous studies have determined that using the euglycemic hyperinsulinemic clamp, a direct measure of insulin sensitivity, this dose of DEX induces whole body insulin resistance (37). Subsequently, hearts were removed for measurement of coronary luminal LPL activity and Western blotting.

Tissue-specific response to insulin. To assess tissue-specific insulin resistance, skeletal muscle (gastrocnemius and soleus from hindleg) and heart from control and 4-h DEX-treated animals were evaluated for total and phospho-IRS-1 (Tyr^{989}) and total and phospho-Akt at Ser^{473} by 10-fold of the basal value (2 mg/kg). For exogenous administration, the dosing with corticosteroids depends on the disease condition and varies from 75 to 300 mg/day (~1–4 mg/kg). Previous studies have determined that using the euglycemic hyperinsulinemic clamp, a direct measure of insulin sensitivity, this dose of DEX induces whole body insulin resistance (37). Subsequently, hearts were removed for measurement of coronary luminal LPL activity and Western blotting.

![Fig. 1. Responses of skeletal muscle and heart to insulin (Ins). Skeletal muscle (gastrocnemius and soleus from hindleg) and heart from control and 4-h dexamethasone (DEX)-treated animals were evaluated for phospho- (p-)IRS-1 (at Tyr^{989}) and total (T)-IRS-1 (A) and p-Akt (at Ser^{473}) and T-Akt (B) before and after 10 min of injecting rapid-acting insulin into the tail vein (8 U Humulin R) using Western blot. Results are means ± SE of 5 rats in each group. *Significantly different from controls (no DEX or insulin); #significantly different from controls given insulin, P < 0.05.](http://ajpendo.physiology.org)
LPL and AMPK gene expression. LPL and AMPK gene expression were measured in the indicated groups using RT-PCR as described previously (37).

Heart tissue homogenization. Hearts from control and DEX rats were cleared of blood with flushing buffer through the aorta, and atria and other tissues were removed. Ventricles were freeze-clamped in liquid nitrogen and stored until total cardiac LPL activity was measured as described previously (39).

Coronary lumen LPL activity. To measure coronary endothelium-bound LPL, hearts were perfused retrogradely by the nonrecirculating Langendorff technique with Krebs-Henseleit buffer containing 10 mM glucose (39). The perfusion solution was changed to buffer containing FA-free BSA (1%) and heparin (5 U/ml). The coronary effluent was collected in timed fractions (10 s) over 5 min and assayed for LPL activity by measuring the hydrolysis of a [3H]triolein substrate emulsion (39, 41).

Western blotting. Western blot was carried out as described previously (24). Measuring the phospho forms of AMPK and p38 MAPK is a surrogate for estimation of their activities. For measuring whole heart LPL protein, we used 5D2, a monoclonal mouse anti-bovine LPL (generously provided by Dr. J. Brunzell, University of Washington, Seattle, WA) and sheep anti-mouse IgG.

Isolated cardiac myocytes. Ventricular calcium-tolerant myocytes were prepared with a previously described procedure (39). To examine the direct influence of DEX and insulin on LPL activity, cardiomyocytes were plated on laminin-coated six-well culture plates (to a density of 200,000 cells/well). Cells were maintained using Medium 199 and incubated at 37°C under an atmosphere of 95% O2-5% CO2 for 16 h. Subsequently, and where indicated, DEX (100 nM) or insulin (100 nM) (16) or LY-294002 (50 μM, a PI 3-kinase inhibitor) was added to the culture medium. Unlike other studies that used high concentrations of insulin (24), 100 nM insulin has no inhibitory effect on AMPK phosphorylation. Following the indicated times, myocyte basal LPL activity released into the medium was measured. To release surface-bound LPL activity, heparin (8 U/ml, 1 min) was added to the culture plates, and aliquots of cell medium were removed, separated by centrifugation, and assayed for LPL activity. In separate experiments, following incubation of plated myocytes (0.4 × 10⁶ cells in a 60 × 15 mm tissue culture dish) with DEX and insulin, myocyte cell lysates were also used for immunoprecipitation and Western blotting. In addition, the effects of DEX plus insulin (DEX+Ins) in myocytes were also determined in the presence or absence of 1 μM cytochalasin D (CTD, an actin polymerization inhibitor) or 50 μM LY-294002 (a PI 3-kinase inhibitor).

Nuclear localization of p38 MAPK. Nuclear localization of p38 MAPK was determined as described previously (23). Using an antibody against Histone H3 as a nuclear marker, we show good purity of nuclear fractions (data not shown).

Filamentous and globular actin. The filamentous (F)-actin/globular (G)-actin ratio in the whole heart and isolated myocytes was determined by Western blotting using an in vivo assay kit.

Immunoprecipitation. Following incubation of plated cardiomyocytes with DEX (100 nM) or DEX+Ins (100 nM), cell lysates were immunoprecipitated using an Akt monoclonal antibody rotating overnight at 4°C. The immunocomplex and the supernatant were resuspended in Laemmli buffer and heated for 5 min at 95°C. The immunocomplex was separated into two equal portions, each of which was immunoblotted with anti-heat shock protein (HSP)25 and anti-Akt.

Silencing of p38 MAPK by siRNA. Small interfering (si)RNA transfection of p38 MAPK in cardiomyocytes was carried out using a kit from Santa Cruz Biotechnology. Briefly, in six-well culture plates, 0.1 × 10⁶ cells were plated and subsequently exposed to the siRNA

Fig. 2. Lipoprotein lipase (LPL) gene expression and protein and activity measurement in hearts from control and DEX-treated animals. With 50 mg of homogenized ventricular tissue from control, and animals given DEX for various intervals, LPL gene and protein (A) and activity (B) were determined. To evaluate LPL exclusively localized at the coronary lumen, hearts from the different groups were also perfused in nonrecirculating retrograde mode with heparin. C: coronary effluents were collected (for 10 s) over 5 min, but only peak LPL activities are illustrated. Results are means ± SE of 5 rats in each group: *Significantly different from untreated controls (0 min); #significantly different from DEX (20–40 min), P < 0.05.
(or scrambled, Scr) solution for 8 h at 37°C in a CO₂ incubator. Following this, the medium was changed to Medium 199, and the cells were incubated for another 16 h. Subsequently, and where indicated, DEX (100 nM)+Ins (100 nM) was added to the culture medium for 8 h, and LPL (released by heparin), p38 MAPK, AMPK, and HSP25 (using Western blotting) were determined.

**Plasma measurements.** Following DEX, blood samples from the tail vein were collected, and blood glucose was determined using a glucometer and glucose test strips (Accu-Chek Advantage, Roche). Diagnostic kits were used to measure TG (Sigma), nonesterified fatty acid (NEFA, Wako), and insulin (Linco).

**Materials.** [3H]triolein (Amersham Canada), heparin sodium injection (1,000 USP U/ml Hapalean; Organon Teknika), F-actin/G-actin kit (Cytoskeleton, Denver, CO). Total AMPKα, phospho-AMPKα (Thr172), p38 MAPK, phospho-p38 MAPK (Thr180/Tyr182), phospho-IRS-1 (Tyr989), phospho-Akt (Ser473), total-IRS-1, and total-Akt antibodies were obtained from Cell Signaling (Danvers, MA). HSP25 and phospho-HSP25 (S86) antibodies were from GeneTex (San Antonio, TX). An ECL detection kit was from Amersham. All other chemicals were obtained from Sigma Chemical.

**Statistical analysis.** Values are means ± SE. Wherever appropriate, one-way ANOVA followed by the Bonferroni test was used to determine differences between group mean values. The level of statistical significance was set at P < 0.05.

**RESULTS**

**General characteristics of the experimental animals.** Chronic DEX treatment induces insulin resistance, hyperinsulinemia, and hyperglycemia (4). In the present study, injection of DEX for 4 h was not associated with either hyperinsulinemia or hyperglycemia (Table 1). However, the euglycemic hyperinsulinemic clamp, a direct measure of insulin sensitivity, revealed that the glucose infusion rate necessary to maintain euglycemia was lower following DEX administration (Table 1). We also evaluated plasma TG and FA at 4 h following DEX. Interestingly, although plasma TG declined, there was no effect on plasma FA (Table 1).

**Tissue-specific insulin resistance.** Whole body insulin resistance could embrace metabolic abnormalities in multiple organs (38). We assessed the effects of DEX on the responses of skeletal muscle and cardiac tissue to insulin. In skeletal muscle,
both basal and insulin-stimulated tyrosine phosphorylation of IRS-1 (Fig. 1A) and Akt (Fig. 1B) were reduced after 4 h of DEX. Unexpectedly, these effects were not observed in cardiac tissue, which demonstrated a normal response to insulin when IRS-1 (Fig. 1A) and Akt (Fig. 1B) phosphorylation were measured. Following 4 h of DEX, total IRS-1 and total Akt (basal and insulin stimulated) did not change in skeletal and cardiac muscle compared with control (Fig. 1, A and B). Thus, in cardiac tissue, any observed changes at 4 h are likely due to a direct effect of DEX and independent of insulin signaling.

Changes in LPL following DEX. LPL-mediated hydrolysis of circulating TG-rich lipoproteins at the coronary lumen provides the heart with FA (35). DEX increased LPL mRNA levels, 60–240 min after injection (Fig. 2A). To determine whether the change in LPL gene following DEX was related to augmented protein synthesis, we measured LPL protein and activity in heart homogenates. Interestingly, the change in gene expression was not reflected in an alteration in whole heart LPL protein (Fig. 2A, immunoblot) or activity (Fig. 2B) up to 240 min after DEX. However, when we measured LPL activity localized to the coronary lumen by retrogradely perfusing isolated hearts with heparin, an increase in LPL activity became apparent as early as 120 min subsequent to injection of DEX (Fig. 2C), and was maintained up to 4 h (Fig. 2C). The DEX-induced increase in heparin-releasable LPL activity at the vascular lumen after 120–240 min was substantial compared with control (∼3-fold; Fig. 2C).

Influence of DEX on cardiac AMPK and p38 MAPK phosphorylation. Previous studies from our laboratory (22) have reported significantly higher AMPK phosphorylation in hearts from streptozotocin-diabetic animals, a model of poorly controlled type 1 diabetes. In the present study, an early (20 min) increase of cardiac AMPK phosphorylation was observed, that peaked at 40 min after injection of DEX (Fig. 3B). With time, AMPK phosphorylation in hearts from DEX-treated animals declined but was still higher than control after 4 h (Fig. 3B). An increase in total AMPK protein became apparent only after 120 min of DEX, with a maximum observed after 4 h (Fig. 3B, immunoblot). This change in AMPK total protein paralleled a rise in AMPK gene expression (Fig. 3A). p38 MAPK is a downstream target of AMPK (29). Estimation of cardiac cytosolic p38 MAPK phosphorylation showed a similar pattern to that seen with activation of AMPK: rapid activation followed by a decline (Fig. 3C). Once phosphorylated, p38 MAPK relocates to the nucleus (48). Separation of the nuclear fraction revealed that, concurrent to the decline in cytosolic p38 MAPK phosphorylation, nuclear p38 MAPK phosphorylation increased (Fig. 3C, immunoblot).

DEX stimulates cardiac actin polymerization. p38 MAPK phosphorylation is suggested to regulate actin polymerization through its phosphorylation of HSP25. In turn, the actin cytoskeleton has been implicated in managing myocyte LPL secretion (15). Marked phosphorylation of HSP25 was evident after 120 min of DEX, an effect that intensified at 4 h (Fig. 4A). To determine whether HSP25 phosphorylation elicits F-actin polymerization, we quantitated F-actin and G-actin cellular fractions by use of Western blot. In the resting cardiomyocyte, the proportion of polymerized F-actin is consistently higher than G-actin (90:10). Additionally, an increase in the F/G-actin ratio indicates actin polymerization. DEX increased the F/G-
actin ratio (Fig. 4B). Interestingly, the increase in F-actin polymerization closely mirrored the enlargement of LPL activity after DEX (Fig. 2C).

In vitro effects of DEX on isolated control myocytes. LPL at the coronary lumen is acquired from underlying cardiomyocytes. In these cells, following their synthesis, LPL is transported onto cell surface HSPG binding sites (34). To directly examine the effects of DEX on myocyte LPL, control cardiomyocytes were incubated with DEX for varying times. In the absence of DEX, no change in AMPK phosphorylation was observed over time (data not shown). Unlike its effects in vivo, DEX-activated AMPK phosphorylation in cardiomyocytes was delayed (becoming apparent only at 60 min) and was maintained up to 8 h after DEX treatment (Fig. 5A). In addition, no change in total AMPK protein was observed (Fig. 5A, immunoblot). The activation of AMPK was temporally related to phosphorylation of cytosolic p38 MAPK (Fig. 5B), which declined over time as a consequence of its nuclear translocation (Fig. 5B, immunoblot). Interestingly, p38 MAPK activation in vitro was not reflected in phosphorylation of HSP25 (Fig. 5C, left), nor was there any evidence of F-actin polymerization (Fig. 5C, right) or an augmentation of heparin-releasable LPL activity up to 4 h after incubation with DEX (Fig. 6A, left).

Insulin mediates the in vitro effects of DEX on cardiomyocyte LPL activity. We evaluated whether insulin could explain the dissimilar effects of DEX observed in vivo and in vitro. Figure 6A (right) illustrates both basal and heparin-releasable LPL activity. Incubation of myocytes with either insulin or DEX alone for up to 8 h had no effect on basal or heparin-releasable LPL activity. The simultaneous addition of DEX with insulin for 4 h also had no influence on this enzyme (data not shown). Interestingly, extending the incubation with DEX and insulin for 8 h appreciably enhanced heparin-releasable activity in the medium (6A, right). This increase in LPL following simultaneous addition of DEX and insulin closely mirrored the robust elevation in both phosphorylation of HSP25 (6B, middle) and F-actin polymerization (6B, right). These data suggest that the effects of DEX on LPL are apparent only in the presence of insulin. In the presence of DEX, HSP25 forms a complex with protein kinase B (Akt) as a means to safeguard this cell survival kinase. As insulin by phosphorylating Akt dissociates HSP25 from its complex with Akt, allowing p38 MAPK to phosphorylate HSP25, induce F-actin polymerization, and eventual LPL translocation to the myocyte cell surface, we examined the association between Akt and HSP25 in the presence of DEX and DEX+Ins. In cardiomyocytes, DEX treatment increased the association between Akt and HSP25, an effect that was reduced in the presence of insulin (Fig. 6B, left). The presence of LY-294002 prevented the dissociation of Akt from HSP25 (Fig. 6B, left) and reduced phospho-HSP25 (Fig. 6B, middle) and F-actin polymerization (Fig. 6B, right). More importantly, LY-294002 reduced the augmented heparin-releasable LPL activity observed with DEX+Ins (Fig. 6A).

To investigate the involvement of the actin cytoskeleton in the DEX+Ins-mediated augmentation of myocyte LPL, myocytes were pretreated with an actin polymerization inhibitor CTD (34) before incubation with DEX+Ins. CTD reduced the effect of DEX+Ins to increase myocyte HR-LPL (heparin-releasable LPL) without any effect on basal activity (Control 2,222 ± 89, DEX+Ins 5,846 ± 126, Dex+Ins+CTD 3,149 ± 186 nmol·h⁻¹·10⁻⁶ cells, P < 0.05).
Silencing of p38 MAPK prevents cardiomyocyte LPL recruitment observed with DEX + Ins. To confirm the relationship between p38 MAPK and LPL, we used short interfering RNA to silence p38 MAPK expression in isolated cardiomyocytes and then treated the cells with DEX + Ins. We first validated successful p38 MAPK inhibition using Western blotting (Fig. 7A, immunoblot). Interestingly, silencing p38 MAPK in myocytes treated with DEX + Ins for 8 h demonstrated a decrease in phosphorylation of HSP25 (Fig. 7B) with a concurrent decline in heparin-releasable LPL activity (Fig. 7C). Silencing of p38 MAPK had no effect on phosphorylation of AMPK, which remained high following Dex + Ins (Fig. 7A, immunoblot).

**DISCUSSION**

Glucocorticoids are widely accepted to cause insufficiency in insulin action, with insulin resistance causing changes in metabolism in multiple organ systems such as skeletal muscle, liver, and adipose tissue (12). In this study, we demonstrate that DEX produced whole body insulin resistance, which was likely accounted for by changes in the responses of skeletal muscle to insulin. In other insulin-sensitive tissues, like the heart, although no changes were observed in insulin signaling, we describe rapid nongenomic alterations in cardiac metabolism following acute DEX treatment. These include phosphorylation of AMPK, p38 MAPK, and HSP25 that cause actin cytoskeleton rearrangement, facilitating LPL translocation to the myocyte cell surface. Transfer of the enzyme from the myocyte cell surface to the coronary lumen would be expected to increase LPL-derived FA provision to the heart, likely compromising glucose utilization (36).

Skeletal muscle accounts for 80% of insulin-induced glucose disposal in the human body (12); thus, it is a major target for glucocorticoid-induced insulin resistance. In skeletal muscle, insulin stimulates glucose uptake, utilization, and storage. As cortisol administration does not alter the number of insulin receptors in skeletal muscle (5), it is likely that glucocorticoids alter glucose metabolism through its post-receptor effects on downstream insulin signaling or glucose utilization. Following chronic DEX treatment, even though its gene expression is unchanged, the phosphorylation of Akt/protein kinase B (PKB) induced by insulin significantly decreases (40). This reduction in insulin signal is paralleled with a decreased glucose uptake and disposal (13). The decreased Akt phosphorylation may be attributed to a decreased insulin receptor tyrosine phosphorylation and IRS protein expression (18). Our study demonstrates that a single dose of DEX also reduces tyrosine phosphorylation...
tion of IRS-1 and phosphorylation of Akt under both basal and insulin-stimulated conditions in skeletal muscle. Surprisingly, unlike skeletal muscle, cardiac tissue displayed normal responses to insulin. At present, whether the heart requires an extended DEX exposure to become insulin resistant is unknown and is under investigation.

The major source of FA for myocardial energy utilization is LPL-mediated hydrolysis of TG-rich lipoproteins at the vascular endothelium. As glucocorticoids have previously been reported to influence the transcription of ~1% of the entire genome in humans (42), we examined cardiac LPL gene expression and establish that acute DEX increases LPL mRNA. However, this change was not coordinated to an increase in LPL protein or activity in whole heart homogenates up to 4 h after DEX treatment. It is possible that at this early time point the change in LPL gene has not yet been translated to an increase in LPL protein and may become apparent if the duration of DEX treatment is extended beyond 4 h. Nevertheless, in the present study we report that DEX increases cardiac luminal LPL activity, a change that was detectable within 2 h of DEX injection and is likely due to novel and rapid non-genomic phosphorylation of stress kinases that increases move-

Fig. 7. Silencing of p38 MAPK by short interfering (si)RNA. siRNA transfection of p38 MAPK in cardiomyocytes was carried out using a kit from Santa Cruz Biotechnology. Plated myocytes were exposed to the siRNA (or scrambled, Scr). Figure depicts transfection efficiency (A, immunoblot). After this, where indicated, DEX (100 nM) + insulin (100 nM) was added to the culture medium for 8 h, and p38 MAPK, phosphorylation of AMPK (A) and HSP25 (B) (using Western blot), and LPL activity were determined (C). Data are means ± SE; n = 5 myocyte preparations from different animals. *Significantly different from control, #significantly different from Dex + Ins (480 min), P < 0.05.
ment of enzyme to the myocyte cell surface and from there to the vascular lumen.

AMPK is the switch that regulates cellular energy during metabolic stress (19). Particularly related to FA, AMPK can regulate FA delivery through its regulation of CD36 (30) and FA oxidation through its effect on acetyl-CoA carboxylase (25). Recently, we have also demonstrated that following AMPK activation heparin-releasable coronary lumen LPL activity is amplified, providing an immediate compensatory response by the heart to guarantee FA supply and ATP production. In addition, in hearts from fasted animals (that demonstrate augmented AMPK phosphorylation and LPL activity), inhibition of AMPK phosphorylation using Ara-A or insulin reduced cardiac luminal LPL activity (1). In the present study, the augmentation in coronary LPL activity was preceded by a rapid (within 20 min) and intense phosphorylation in AMPK that was not sustainable and decreased over time. The early increase in AMPK phosphorylation may well be a product of the rapid action of glucocorticoids to compromise ATP production that highlights its actions as an immunosuppressant to reduce inflammatory processes (44). The gradual decline in AMPK activation is likely a consequence of the excessive amount of cardiac LPL-derived FA. Cardiac LPL is a major determinant of plasma TG (28), and the increase in cardiac luminal LPL is associated with a decline in circulating TG and an increase in cardiac FA (palmitic and oleic acid) and TG levels (36). High FA or TG, through their formation of ceramide, has been shown to activate protein phosphatase 2A, leading to dephosphorylation of AMPK (46). As AMPK phosphorylation following prolonged DEX never declined to control levels, it is possible that, 2–4 h after DEX, AMPK phosphorylation is a balance between FA inhibition of AMPK (23) and activation of the AMPK gene, resulting in higher total AMPK protein.

Given the delay between AMPK activation (within minutes) and the increase in coronary lumen LPL activity (within hours), we considered the possibility that the early activation of AMPK may have turned on other downstream signals. One downstream target of AMPK is p38 MAPK, and there was coincident activation of both AMPK and p38 MAPK following injection of DEX. Other studies have demonstrated that AMPK activates p38 MAPK through its interaction with transforming growth factor-β-activated protein kinase-1-binding protein-1 (29). Cytosolic activation of p38 MAPK results in its transfer to the nucleus and gene activation through a number of transcription factors (45). In the nucleus, p38 MAPK can also activate MAPKAP kinase-2, which is then exported to phosphorylate HSP25. Our studies in the heart confirmed that cytosolic activation of p38 MAPK was followed by its nuclear translocation. More importantly, with increasing duration of DEX, phosphorylation of HSP25 progressively increased. HSP25 is known to inhibit actin polymerization, and its phos-

Fig. 8. Schema of mechanisms that are likely involved in regulating the effects of DEX and insulin on cardiac LPL.
phorylation results in a decline of this inhibitory function (11). In this setting, actin monomers are released from the phosphorylated HSP25 to self-associate to form fibrillar actin. We (34) and others (15) have reported actin cytoskeleton reorganization within the myocyte as an important means by which LPL is secreted onto plasma membrane HSPG binding sites. Since the increase in luminal LPL activity at 2 and 4 h after DEX corresponded to an enlargement in the F-actin/G-actin ratio, our data suggest that AMPK and p38 MAPK, through their control of HSP25 and the actin cytoskeleton act in unison to facilitate LPL translocation to the myocyte cell surface and ultimately to the coronary lumen. The importance of p38 MAPK in modulating the effects of DEX on myocyte LPL was evident in our silencing experiment, where p38 MAPK siRNA prevented HSP25 phosphorylation and an increase in LPL activity (in the absence of any change in AMPK phosphorylation).

Recently, observations from our laboratory have also shown that throbmin activates p38 MAPK and increases myocyte LPL activity and that myocytes preincubated with a p38 MAPK inhibitor (SB-202190, 20 μM) abolished these effects of thrombin (23).

In the heart, LPL is synthesized in the underlying myocytes before it is translocated to the luminal side of the coronary vessel wall with the help of heparan sulfate oligosaccharides acting as extracellular chaperones (33). We next evaluated the direct effects of DEX on stress kinases and their control of cardiomyocyte LPL. Unlike our in vivo data, although 100 nM DEX activated AMPK in vitro, the pattern of AMPK phosphorylation was different. In cardiomyocytes, AMPK stimulation occurred later (60 min), with no falling off over time. At present, an explanation for such effects is unknown. It is possible that the limited demand for energy in nonbeating quiescent myocytes delays the activation of AMPK, whereas the absence of FA in the myocyte incubation medium prevents the gradual decline in AMPK observed in vivo. Activation of AMPK by DEX in isolated myocytes was associated with cytosolic phosphorylation of p38 MAPK followed by its nuclear transfer. However, no phosphorylation of HSP25 was observed in this setting; nor was there any actin polymerization, suggesting that HSP25 phosphorylation requires additional elements. In mouse embryonic fibroblasts, HSP25 associates with Akt to form an oligomer, and activation of Akt releases HSP25 from its complex with Akt, facilitating HSP25 phosphorylation, dissociation of the actin dimers and monomers (48), and, ultimately, actin polymerization. It is possible that in the presence of DEX the cellular response of the heart to evade cell death is for HSP25 to form a complex with Akt as a means to safeguard this cell survival kinase. Indeed, in cardiomyocytes incubated with DEX alone, there was a robust association between Akt and HSP25. Our data suggest that insulin, by phosphorylating Akt, dissociates HSP25 from its complex with Akt, allowing p38 MAPK to phosphorylate HSP25 and induce F-actin polymerization, and eventually the translocation of LPL to the myocyte cell surface (Fig. 8). A previous study has also reported that the increase in LPL activity following DEX is observed only with the prolonged exposure of myocytes to both insulin and DEX (15).

In summary, following acute administration of DEX, it is LPL recruitment to the coronary lumen that supports an augmented FA supply to the heart. The mechanism underlying this process embraces activation of stress kinases like AMPK and p38 MAPK, a requirement of insulin, and an increase in actin cytoskeleton polymerization. As elevated FA use has been implicated in a number of metabolic, morphological, and mechanical changes and more recently, in “lipotoxicity”, our data suggest that enlargement in LPL at the cardiomyocyte cell surface and likely transfer to the vascular lumen could play a crucial role in the development of heart disease when glucocorticoids are used chronically.

GRANTS

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REFERENCES


